SHOUT
SHARE
IT

THE
BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

Editorial Board

JOHN B. BUCK, National Institutes of Health
JOHN O. CORLISS, University of Maryland
DONALD P. COSTELLO, Woods Hole, Massachusetts
JOHN D. COSTLOW, Duke University
PHILIP B. DUNHAM, Syracuse University
CATHERINE HENLEY, National Institutes of Health
GEORGE O. MACKIE, University of Victoria
HOWARD A. SCHNEIDERMAN, University of California, Irvine
RALPH I. SMITH, University of California, Berkeley
GROVER C. STEPHENS, University of California, Irvine
CARROLL M. WILLIAMS, Harvard University
EDWARD O. WILSON, Harvard University

W. D. RUSSELL-HUNTER, Syracuse University
Managing Editor

VOLUME 151
JULY TO DECEMBER, 1976

Printed and Issued by
LANCASTER PRESS, Inc.
PRINCE & LEMON STS.
LANCASTER, PA.

THE BIOLOGICAL BULLETIN is issued six times a year at the Lancaster Press, Inc., Prince and Lemon Streets, Lancaster, Pennsylvania.

Subscriptions and similar matter should be addressed to The Biological Bulletin, Marine Biological Laboratory, Woods Hole, Massachusetts. Agent for Great Britain: Wheldon and Wesley, Limited, 2, 3 and 4 Arthur Street, New Oxford Street, London, W. C. 2. Single numbers, $8.00. Subscription per volume (three issues), $22.00.

Communications relative to manuscripts should be sent to Dr. W. D. Russell-Hunter, Marine Biological Laboratory, Woods Hole, Massachusetts 02543 between June 1 and September 1, and to Dr. W. D. Russell-Hunter, P.O. Box 103, University Station, Syracuse, New York 13210, during the remainder of the year.

Second-class-postage paid at Lancaster, Pa.

LANCASTER PRESS, INC., LANCASTER, PA.

CONTENTS

No. 1, August, 1976

Annual Report of the Marine Biological Laboratory 1

Cooley, Lynn, Dana R. Crawford and Stephen H. Bishop
Urease from the lugworm, *Arenicola cristata* 96

Elvin, David W.
Seasonal growth and reproduction of an intertidal sponge *Haliclona permollis* (Bowerbank) 108

Forward, Richard B., Jr.
A shadow response in a larval crustacean 126

Gwilliam, G. F.
The mechanism of the shadow reflex in Cirripedia. III. Rhythmical patterned activity in central neurons and its modulation by shadows 141

Kahler, George A., Frank M. Fisher, Jr., and Ronald L. Sass
The chemical composition and mechanical properties of the hinge ligament in bivalve molluscs 161

Mackie, George O., C. L. Singla, and Catherine Thiriot-Quiévreux
Nervous control of ciliary activity in gastropod larvae 182

McLaren, Ian A.
Inheritance of demographic and production parameters in the marine copepod *Eurytemora herdmani* 200

Mills, Claudia E.
Podocoryne selena, a new species of hydroid from the Gulf of Mexico, and a comparison with *Hydractinia echinata* 214

Pardy, R. L.
The production of aposymbiotic hydra by the photodestruction of green hydra zoochlorellae 225

Sweeney, Beatrice M.
Circadian rhythms in corals, particularly Fungiidae 236

Timko, Patricia L.
Sand dollars as suspension feeders: a new description of feeding in *Dendraster excentricus* 247

Tweedell, Kenyon S.
Utilization of ^{3}H-thymidine triphosphate by developing stages of *Pectinaria gouldii* 260

No. 2, October, 1976

Ache, B. W., Z. M. Fuzessery, and W. E. S. Carr
Antennular chemosensitivity in the spiny lobster, *Panulirus argus:* comparative tests of high and low molecular weight stimulants 273

Chevone, Boris I. and A. Glenn Richards
Ultrastructure of the atypic muscles associated with terminalial inversion in male *Aedes aegypti* (L.) 283

FISHER, THOMAS R.
Oxygen uptake of the solitary tunicate *Styela plicata* 297
HARZA, T. AND LENKE MÁTYÁS
Seasonal variations of sodium and potassium concentrations in different parts of the frog myocardium 306
HERNANDORENA, A.
Effects of temperature on the nutritional requirements of *Artemia salina* (L.) 314
HUNTER, F. R.
Permeability of trout erythrocytes to nonelectrolytes 322
JØRGENSEN, C. BARKER
Comparative studies on the function of gills in suspension feeding bivalves, with special reference to effects of serotonin 331
LEVERSEE, GORDON J.
Flow and feeding in fan-shaped colonies of the gorgonian coral, *Leptogorgia* 344
NIMITZ, SR. M. AQUINAS
Histochemical changes in gonadal nutrient reserves correlated with nutrition in the sea stars, *Pisaster ochraceus* and *Patiria miniata* 357
SCHULTZ, T. WAYNE AND JOHN R. KENNEDY
Cytotoxic effects of the herbicide 3-amino-1,2,4-triazole on *Daphina pulex* (Crustacea: Cladocera) 370
THOMAS, J. D., M. POWLES, AND R. LODGE
The chemical ecology of *Biomphalaria glabrata:* the effects of ammonia on the growth rate of juvenile snails 386
ABSTRACTS OF PAPERS PRESENTED AT THE MARINE BIOLOGICAL LABORATORY 398

NO. 3, DECEMBER, 1976

ANDERSON, O. ROGER AND ALLAN W. H. BÉ
A cytochemical fine structure study of phagotrophy in a planktonic foraminifer, *Hastigerina pelagica* (d'Orbigny) 437
DURAND, JACQUES P.
Ocular development and involution in the European cave salamander, *Proteus anguinus* Laurenti 450
DURLIAT, MICHELE AND ROGER VRANCKX
Coagulation in the crayfish, *Astacus leptodactylus:* attempts to identify a fibrinogen-like factor in the hemolymph 467
HOFFMANN, RICHARD J.
Genetics and asexual reproduction of the sea anemone *Metridium senile* 478
JENNINGS, J. B. AND S. R. GELDER
Observations on the feeding mechanism, diet and digestive physiology of *Histriobdella homari* van Beneden 1858: an aberrant polychaete symbiotic with North American and European lobsters 489
KASTENDIEK, JON
Behavior of the sea pansy *Renilla kollikeri* Pfeffer (Coelenterata: Pennatulacea) and its influence on the distribution and biological interactions of the species 518

McCarthy, J. F., A. N. Sastry and G. C. Tremblay
Thermal compensation in protein and RNA synthesis during the intermolt cycle of the American lobster, *Homarus americanus* 538
Mitton, Jeffry B. and Richard K. Koehn
Morphological adaptation to thermal stress in a marine fish, *Fundulus heteroclitus* 548
Oguro, Chitaru, Miéko Komatsu and Yasuo T. Kano
Development and metamorphosis of the sea star, *Astropecten scoparius* Valenciennes 560
Ruby, E. G. and K. H. Nealson
Symbiotic association of *Photobacterium fischeri* with the marine luminous fish *Monocentris japonica:* a model of symbiosis based on bacterial studies 574
Smith, Ralph I.
Exchanges of sodium and chloride at low salinities by *Nereis diversicolor* (Annelida, Polychaeta) 587
Turgeon, Kenneth W.
Osmotic adjustment in an estuarine population of *Urosalpinx cinerea* (Say, 1822) (Muricidae, Gastropoda) 601
Index to Volume 151 615

Volume 151 Number 1

THE
BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

Editorial Board

JOHN B. BUCK, National Institutes of Health
JOHN O. CORLISS, University of Maryland
DONALD P. COSTELLO, Woods Hole, Massachusetts
JOHN D. COSTLOW, Duke University
PHILIP B. DUNHAM, Syracuse University
CATHERINE HENLEY, National Institutes of Health
GEORGE O. MACKIE, University of Victoria
HOWARD A. SCHNEIDERMAN, University of California, Irvine
RALPH I. SMITH, University of California, Berkeley
GROVER C. STEPHENS, University of California, Irvine
CARROLL M. WILLIAMS, Harvard University
EDWARD O. WILSON, Harvard University

W. D. RUSSELL-HUNTER, Syracuse University
Managing Editor

AUGUST, 1976

Marine Biological Laboratory
LIBRARY
AUG [illegible] 97
Woods Hole, Mass.

Printed and Issued by
LANCASTER PRESS, Inc.
PRINCE & LEMON STS.
LANCASTER, PA.

THE BIOLOGICAL BULLETIN

THE BIOLOGICAL BULLETIN is issued six times a year at the Lancaster Press, Inc., Prince and Lemon Streets, Lancaster, Pennsylvania.

Subscriptions and similar matter should be addressed to THE BIOLOGICAL BULLETIN, Marine Biological Laboratory, Woods Hole, Massachusetts. Agent for Great Britain: Wheldon and Wesley, Limited, 2, 3 and 4 Arthur Street, New Oxford Street, London, W. C. 2. Single numbers, $7.00. Subscription per volume (three issues), $18.00, (this is $36.00 per year for six issues).

Communications relative to manuscripts should be sent to Dr. W. D. Russell-Hunter, Marine Biological Laboratory, Woods Hole, Massachusetts 02543 between May 23 and September 1, and to Dr. W. D. Russell-Hunter, P.O. Box 103, University Station, Syracuse, New York 13210, during the remainder of the year.

Copyright © 1976, by the Marine Biological Laboratory
Second-class postage paid at Lancaster, Pa.

INSTRUCTIONS TO AUTHORS

THE BIOLOGICAL BULLETIN accepts original research reports of intermediate length on a variety of subjects of biological interest. In general, these papers are either of particular interest to workers at the Marine Biological Laboratory, or of outstanding general significance to a large number of biologists throughout the world. Normally, review papers (except those written at the specific invitation of the Editorial Board), very short papers (less than five printed pages), preliminary notes, and papers which describe only a new technique or method without presenting substantial quantities of data resulting from the use of the new method cannot be accepted for publication. A paper will usually appear within four months of the date of its acceptance.

The Editorial Board requests that manuscripts conform to the requirements set below; those manuscripts which do not conform will be returned to authors for correction before review by the Board.

1. ***Manuscripts.*** Manuscripts must be typed in double spacing (*including* figure legends, foot-notes, bibliography, etc.) on one side of 16- or 20-lb. bond paper, 8½ by 11 inches. They should be carefully proof-read before being submitted and all typographical errors corrected legibly in black ink. Pages should be numbered. A left-hand margin of at least 1½ inches should be allowed.

2. ***Tables, Foot-Notes, Figure Legends, etc.*** Tables should be typed on separate sheets and placed in correct sequence in the text. Because of the high cost of setting such material in type, authors are earnestly requested to limit tabular material as much as possible. Similarly, foot-notes to tables should be avoided wherever possible. If they are essential, they should be indicated by asterisks, daggers, etc., rather than by numbers. Foot-notes are not normally permitted in the body of the text. Such material should be incorporated into the text where appropriate. Explanations of figures should be typed double-spaced and placed on separate sheets at the end of the paper.

3. ***A condensed title*** or running head of no more than 35 letters and spaces should be included.

Continued on Cover Three

Marine Biological Laboratory
LIBRARY
AUG 30 1976
Woods Hole, Mass.

NOTICE TO SUBSCRIBERS

Effective with the first issue of Volume 152 (February, 1977), the subscription price for THE BIOLOGICAL BULLETIN will be raised. Subscription per volume (three issues) will be $22.00 (this is $44.00 per year for six issues); single numbers will be $8.00 each. Continuing 1977 subscriptions for which payment has actually been received before November 1, 1976 will be honored at the old rate.

Vol. 151, No. 1 August, 1976

THE
BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

THE MARINE BIOLOGICAL LABORATORY

Seventy-eighth Report, for the Year 1975—Eighty-eighth Year

I. Trustees and Executive Committee (as of August, 1975) 1
II. Certificate of Organization 5
III. Articles of Amendment 6
IV. Bylaws of the Corporation 6
V. Report of the Director 10

Addenda:

1. Memorial 16
2. The Staff 17
3. Investigators, Fellowships, and Students 34
4. Fellows and Scholarships 47
5. Training Programs 48
6. Tabular View of Attendance, 1971–1975 50
7. Institutions Represented 51
8. Friday Evening Lectures 53
9. Members of the Corporation 54

VI. Report of the Librarian 85
VII. Report of the Treasurer 85

I. TRUSTEES

Including Action of 1975 Annual Meeting

Denis M. Robinson, *Chairman of the Board of Trustees*, High Voltage Engineering Corporation, Burlington, Massachusetts 01803

Gerard Swope, Jr., *Honorary Chairman of the Board of Trustees*, Croton on-Hudson, New York, New York 10520

Alexander T. Daignault, *Treasurer*, 1114 Avenue of the Americas, New York, New York 10005

James D. Ebert, *President of the Corporation*, Carnegie Institution of Washington, Baltimore, Maryland 21210

Keith R. Porter, *Director*, University of Colorado, Boulder, Colorado 80302

David Shepro, *Clerk of the Corporation*, Boston University, Boston, Massachusetts 02215

Copyright © 1976, by the Marine Biological Laboratory
Library of Congress Card No. A38-518

EMERITI

William R. Amberson, Falmouth, Massachusetts
Phillip B. Armstrong, State University of New York, Upstate Medical Center
Eric G. Ball, Marine Biological Laboratory
Dugald E. S. Brown, Placida, Florida
Kenneth S. Cole, National Institutes of Health
Donald P. Costello, University of North Carolina
Paul S. Galtsoff, Woods Hole, Massachusetts
Harry Grundfest, Columbia University, College of Physicians and Surgeons
Douglas Marsland, Marine Biological Laboratory
Harold H. Plough, Amherst, Massachusetts
C. Ladd Prosser, University of Illinois
A. C. Redfield, Woods Hole, Massachusetts
Carl C. Speidel, University of Virginia
H. Burr Steinbach, Woods Hole, Massachusetts
Albert Szent-Györgyi, Marine Biological Laboratory
W. Randolph Taylor, University of Michigan

CLASS OF 1979

Robert D. Allen, Dartmouth College
Francis D. Carlson, Johns Hopkins University
Hays Clark, Avon Products, Incorporated
Dennis Flanagan, Scientific American
Philip Grant, University of Oregon
Catherine Henley, National Institutes of Health
E. Swift Lawrence, Falmouth National Bank
John W. Moore, Duke University Medical Center
Melvin Spiegel, Dartmouth College

CLASS OF 1978

Michael V. L. Bennett, Albert Einstein College of Medicine
John E. Dowling, Harvard University
Prosser Gifford, Woodrow Wilson Center, Washington, D.C.
Ellen R. Grass, The Grass Foundation
Harlyn O. Halvorson, Brandeis University
Ruth Hubbard, Harvard University
John P. Kendall, Boston, Massachusetts
James W. Lash, University of Pennsylvania
Annemarie Weber, University of Pennsylvania

CLASS OF 1977

Joel E. Brown, State University of New York at Stony Brook
John B. Buck, National Institutes of Health
James Case, University of California, Santa Barbara
William T. Golden, New York, New York
Shinya Inoué, University of Pennsylvania
Stephen W. Kuffler, Harvard Medical School
George D. Pappas, Albert Einstein College of Medicine
Malcolm S. Steinberg, Princeton University
Raymond E. Stephens, Brandeis University and the Marine Biological Laboratory

CLASS OF 1976

Everett Anderson, Harvard Medical School
Frank M. Child, Trinity College, Hartford
George H. A. Clowes, Jr., Harvard Medical School
Philip B. Dunham, Syracuse University
Timothy H. Goldsmith, Yale University
Robert K. Josephson, University of California, Irvine
Lionel I. Rebhun, University of Virginia
Andrew Szent-Györgyi, Brandeis University
Edward O. Wilson, Harvard University

STANDING COMMITTEES

Executive Committee of the Board of Trustees

Denis M. Robinson, *ex officio*
Alexander T. Daignault, *ex officio*
James D. Ebert, *ex officio*
Keith R. Porter, *ex officio*
Harlyn O. Halvorson, 1976
James F. Case, 1976
James W. Lash, 1977
Andrew Szent-Györgyi, 1977
John E. Dowling, 1978
Timothy H. Goldsmith, 1978

Library Committee

David Shepro, *Chairman*
John M. Arnold
Dean Bumpus
Elizabeth Bunce
Jane Fessenden, *ex officio*
James Oschman
Irwin Sherman
Ivan Valiela
Annemarie Weber
George Woodwell, *ex officio*
Donald Zinn

Research Services Committee

Donald T. Frazier, *Chairman*
Francis P. Bowles, *ex officio*
J. A. Hancock, *ex officio*
Anthony Liuzzi
E. F. MacNichol, Jr., *ex officio*
T. Narahashi
George Pappas
Robert V. Rice
Jerome Schiff
Bruce Szamier
Andrew Szent-Györgyi
Seymour Zigman

Supply Department Committee

Sears Crowell, *Chairman*
Francis P. Bowles, *ex officio*
Tom Humphreys
Cyrus Levinthal
E. F. MacNichol, Jr., *ex officio*
Robert Prendergast
John S. Rankin
W. D. Russell-Hunter
Melvin Spiegel
Carl P. Swanson
John Valois, *ex officio*
George Woodwell, *ex officio*

Instruction Committee

Benjamin Kaminer, *Chairman*
Francis D. Carlson
Esther Goudsmit
Arthur Humes
Frederick Lang
E. F. MacNichol, Jr., *ex officio*
Ralph Quatrano
Joel Rosenbaum
J. Richard Whittaker
E. O. Wilson
George Woodwell, *ex officio*

Buildings and Grounds Committee

Francis Hoskin, *Chairman*
Clelmer Bartell
Lawrence B. Cohen
James Green
Robert Gunning, *ex officio*
Audrey E. V. Haschemeyer
Lee V. Leak
E. F. MacNichol, Jr., *ex officio*
Leonard Nelson
Robert D. Prusch
Ronald Przybylski
Leon P. Weiss

Radiation Committee

George T. Reynolds, *Chairman*
Eugene Bell
Francis P. Bowles, *ex officio*
Anthony Liuzzi
Laszlo Lorand
E. F. MacNichol, Jr., *ex officio*
James Morin
Raymond Stephens

Research Space Committee

Joel Brown, *Chairman*
Gary Borisy
Philip Dunham
Philip Grant
Ruth Hubbard
Holger Jannasch
E. F. MacNichol, Jr., *ex officio*
John W. Moore
Raymond Stephens
George Woodwell, *ex officio*

Committee for the Nomination of Officers

Harlyn O. Halvorson
James F. Case
James W. Lash
Andrew Szent-Györgyi
John E. Dowling
Timothy H. Goldsmith

Housing, Food Service and Day Care Committee

Judith Grassle, *Chairman*
Samuel I. Beale
Noel deTerra
Brian M. Salzberg
Homer P. Smith, *ex officio*
Ann Stuart
Leon P. Weiss
Jane Zakevicius

Computer Service Committee

John W. Moore, *Chairman*
Daniel Botkin
Francis P. Bowles
Fred Dodge
Cyrus Levinthal
E. F. MacNichol, Jr., *ex officio*
Melvin Rosenfeld, Jr.
Norman B. Rushforth

Finance Committee

Alexander T. Daignault, *Chairman*
Robert D. Allen
Samuel Lenher
John W. Moore

Safety Committee

Robert Gunning, *Chairman*
Francis P. Bowles
Manuel P. Dutra
Lewis Lawday
Donald Lehy
E. F. MacNichol, Jr.
Raymond Stephens
Frederick E. Thrasher

EMPLOYEE RELATIONS COMMITTEE

JANE FESSENDEN, *Chairman*
JUDITH GRASSLE
LEWIS LAWDAY
DONALD LEHY
ALAN LUNN
THERESA MCKEE
RAYMOND STEPHENS

II. CERTIFICATE OF ORGANIZATION

(On File in the Office of the Secretary of the Commonwealth)

No. 3170

We, Alpheus Hyatt, President, William Stanford Stevens, Treasurer, and William T. Sedgwick, Edward G. Gardiner, Susan Mims and Charles Sedgwick Minot being a majority of the Trustees of the Marine Biological Laboratory in compliance with the requirements of the fourth section of chapter one hundred and fifteen of the Public Statutes do hereby certify that the following is a true copy of the agreement of association to constitute said Corporation, with the names of the subscribers thereto:-

We, whose names are hereto subscribed, do, by this agreement, associate ourselves with the intention to constitute a Corporation according to the provisions of the one hundred and fifteenth chapter of the Public Statutes of the Commonwealth of Massachusetts, and the Acts in amendment thereof and in addition thereto.

The name by which the Corporation shall be known is THE MARINE BIOLOGICAL LABORATORY.

The purpose for which the Corporation is constituted is to establish and maintain a laboratory or station for scientific study and investigations, and a school for instruction in biology and natural history.

The place within which the Corporation is established or located is the city of Boston within said Commonwealth.

The amount of its capital stock is none.

In Witness Whereof, we have hereunto set our hands, this twenty seventh day of February in the year eighteen hundred and eighty-eight, Alpheus Hyatt, Samuel Mills, William T. Sedgwick, Edward G. Gardiner, Charles Sedgwick Minot, William G. Farlow, William Stanford Stevens, Anna D. Phillips, Susan Mims, B. H. Van Vleck.

That the first meeting of the subscribers to said agreement was held on the thirteenth day of March in the year eighteen hundred and eighty-eight.

In Witness Whereof, we have hereunto signed our names, this thirteenth day of March in the year eighteen hundred and eighty-eight, Alpheus Hyatt, President, William Stanford Stevens, Treasurer, Edward G. Gardiner, William T. Sedgwick, Susan Mims, Charles Sedgwick Minot.

(Approved on March 20, 1888 as follows:

I hereby certify that it appears upon an examination of the within written certificate and the records of the corporation duly submitted to my inspection, that the requirements of sections one, two and three of chapter one hundred and fifteen, and sections eighteen, twenty and twenty-one of chapter one hundred and six, of the Public Statutes, have been complied with and I hereby approve said certificate this twentieth day of March A.D. eighteen hundred and eighty-eight.

CHARLES ENDICOTT
Commissioner of Corporations)

III. ARTICLES OF AMENDMENT

(On file in the office of the Secretary of the Commonwealth)

We, James D. Ebert, President, and David Shepro, Clerk of the Marine Biological Laboratory, located at Woods Hole, Massachusetts 02543, do hereby certify that the following amendment to the Articles of Organization of the Corporation was duly adopted at a meeting held on August 15, 1975, as adjourned to August 29, 1975, by vote of 444 members, being at least two-thirds of its members legally qualified to vote in meetings of the corporation:

VOTED: That the Certificate of Organization of this corporation be and it hereby is amended by the addition of the following provisions:

> "No Officer, Trustee or Corporate Member of the corporation shall be personally liable for the payment or satisfaction of any obligation or liabilities incurred as a result of, or otherwise in connection with, any commitments, agreements, activities or affairs of the corporation.
>
> "Except as otherwise specifically provided by the Bylaws of the corporation, meetings of the Corporate Members of the corporation may be held anywhere in the United States.
>
> "The Trustees of the corporation may make, amend or repeal the Bylaws of the corporation in whole or in part, except with respect to any provisions thereof which shall by law, this Certificate or the Bylaws of the corporation, require action by the Corporate Members."

The foregoing amendment will become effective when these articles of amendment are filed in accordance with Chapter 180, Section 7 of the General Laws unless these articles specify, in accordance with the vote adopting the amendment, a later effective date not more than thirty days after such filing, in which event the amendment will become effective on such later date.

In Witness whereof and Under the Penalties of Perjury, we have hereto signed our names this 2nd day of September, in the year 1975, James D. Ebert, President; David Shepro, Clerk.

(Approved on October 24, 1975, as follows:

I hereby approve the within articles of amendment and, the filing fee in the amount of $10 having been paid, said articles are deemed to have been filed with me this 24th day of October, 1975.

PAUL GUZZI
Secretary of the Commonwealth)

IV. BYLAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

(Revised February 13, 1976)

I. (A) The name of the Corporation shall be The Marine Biological Laboratory. The Corporation's purpose shall be to establish and maintain a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history.

(B) Marine Biological Laboratory admits students without regard to race, color, sex, national and ethnic origin to all the rights, privileges, programs and activities generally accorded or made available to students in its courses. It does not discriminate on the basis of race, color, sex, national and ethnic origin in employment, administration of its educational policies, admissions policies, scholarship and other programs.

II. (A) The members of the Corporation ("Members") shall consist of persons elected by the Board of Trustees, upon such terms and conditions and in accordance with such procedures, not inconsistent with law or these bylaws, as may be determined by said Board of Trustees. Except as provided below, any Member may vote at any meeting, either in person or by proxy executed no more than six months prior to the date of such meeting. Members shall serve until their death or resignation unless earlier removed, with or without cause, by the affirmative vote of two-thirds of the Trustees then in office. Any Member who has attained the age of seventy years or has retired from his home institution shall automatically be designated a Life Member provided he signifies his wish to retain his membership. Life Members shall not have the right to vote and shall not be assessed for dues.

(B) The Associates of the Marine Biological Laboratory shall be an unincorporated group of persons (including associations and corporations) interested in the Laboratory and shall be organized and operated under the general supervision and authority of the Trustees.

III. The officers of the Corporation shall consist of a Chairman of the Board of Trustees, President, Director, Treasurer and Clerk, elected or appointed by the Trustees as set forth in Article IX.

IV. The Annual Meeting of the Members shall be held on the Friday following the Second Tuesday in August in each year at the Laboratory in Woods Hole, Massachusetts, at 9:30 a.m. Subject to the provisions of Article VIII(2), at such meeting the Members shall choose by ballot six Trustees to serve four years, and shall transact such other business as may properly come before the meeting. Special meetings of the Members may be called by the Chairman or Trustees to be held at such time and place as may be designated.

V. Twenty-five Members shall constitute a quorum at any meeting. Except as otherwise required by law or these bylaws, the affirmative vote of a majority of the Members voting in person or by proxy at a meeting attended by a quorum (present in person or by proxy) shall constitute action on behalf of the Members.

VI. (A) Inasmuch as the time and place of the Annual Meeting of Members are fixed by these bylaws, no notice of the Annual Meeting need be given. Notice of any special meeting of Members, however, shall be given by the Clerk by mailing notice of the time and place and purpose of such meeting, at least 15 days before such meeting, to each Member at his or her address as shown on the records of the Corporation.

(B) Any meeting of the Members may be adjourned to any other time and place by the vote of a majority of those Members present or represented at the meeting, whether or not such Members constitute a quorum. It shall not be necessary to notify any Member of any adjournment.

VII. The Annual Meeting of the Trustees shall be held promptly after the Annual Meeting of the Corporation at the Laboratory in Woods Hole, Massachusetts. Special meetings of the Trustees shall be called by the Chairman, the President, or by any seven Trustees, to be held at such time and place as may be designated. Notice of Trustees' meetings may be given orally, by telephone, telegraph or in writing; and notice given in time to enable the Trustees to attend, or in any case notice sent by mail or telegraph to a Trustee's usual or last known place of residence, at least one week before the meeting shall be sufficient. Notice of a meeting need not be given to any Trustee if a written waiver of notice, executed by him before or after the meeting is filed with the records of the meeting, or if he shall attend the meeting without protesting prior thereto or at its commencement the lack of notice to him.

VIII. (A) There shall be four groups of Trustees:

(1) Trustees (the "Corporate Trustees") elected by the Members according to such procedures, not inconsistent with these bylaws, as the Trustees shall have determined. Except as provided below, such Trustees shall be divided into four classes of six, one class to be elected each year to serve for a term of four years. Such classes shall be designated by the year of expiration of their respective terms.

(2) Trustees ("Board Trustees") elected by the Trustees then in office according to such procedures, not inconsistent with these bylaws, as the Trustees shall have determined. Except as provided below, such Board Trustees shall be divided into four classes of three, one class to be elected each year to serve for a term of four years. Such classes shall be designated by the year of expiration of their respective terms. It is contemplated that, unless otherwise determined by the Trustees for good reason, Board Trustees shall be individuals who have not been considered for election as Corporate Trustees.

(3) Trustees ex officio, who shall be the Chairman, the President, the Director, the Treasurer, and the Clerk.

(4) Trustees emeriti who shall include any Member who has attained the age of seventy years (or the age of sixty-five and has retired from his home institution) and who has served a full elected term as a regular Trustee, provided he signifies his wish to serve the Laboratory in that capacity. Any Trustee who qualifies for emeritus status shall continue to serve as a regular Trustee until the next Annual Meeting whereupon his office as regular Trustee shall become vacant and be filled by election by the Members or by the Board, as the case may be. The Trustees ex officio and emeriti shall have all the rights of the Trustees, except that Trustees emeriti shall not have the right to vote.

(B) The aggregate number of Corporate Trustees and Board Trustees elected in any year (excluding Trustees elected to fill vacancies which do not result from expiration of a term) shall not exceed nine. The number of Board Trustees so elected shall not exceed three and unless otherwise determined by vote of the Trustees, the number of Corporate Trustees so elected shall not exceed six.

(C) The Trustees and Officers shall hold their respective offices until their successors are chosen in their stead.

(D) Any Trustee may be removed from office at any time with or without cause, by vote of a majority of the Members entitled to vote in the election of Trustees; or for cause, by vote of two-thirds of the Trustees then in office. A Trustee may be removed for cause only if notice of such action shall have been given to all of the Trustees or Members entitled to vote, as the case may be, prior to the meeting at which such action is to be taken and if the Trustee so to be removed shall have been given reasonable notice and opportunity to be heard before the body proposing to remove him.

(E) Any vacancy in the number of Corporate Trustees, however arising, may be filled by the Trustees then in office unless and until filled by the Members at the next Annual Meeting. Any vacancy in the number of Board Trustees may be filled by the Trustees.

(F) A Corporate Trustee or a Board Trustee who has served an initial term of at least 2 years duration shall be eligible for re-election to a second term, but shall be ineligible for re-election to any subsequent term until two years have elapsed after he last served as a Trustee.

IX. The Trustees shall have the control and management of the affairs of the Corporation. They shall elect a Chairman of the Board of Trustees who shall be elected annually and shall serve until his successor is selected and qualified and who shall also preside at meetings of the Corporation. They shall elect a President of the Corporation who shall also be the Vice Chairman of the Board of Trustees and Vice Chairman of meetings of the Corporation, and who shall be elected annually and shall serve until his successor is selected and qualified. They shall elect a Treasurer and Clerk to serve one year, and Board Trustees as described in Article VIII (B). They shall appoint a Director of the Laboratory for a term not to exceed five years, provided the term shall not exceed one year if the candidate has attained the age of 65 years prior to the date of the appointment. They may choose such other officers and agents as they may think best. They may fix the compensation and define the duties of all the officers and agents of the Corporation and may remove them at any time. They may fill vacancies occurring in any of the offices. The Board of Trustees shall have the power to choose an Executive Committee from their own number as provided in Article X, and to delegate to such Committee such of their own powers as they may deem expedient in addition to those powers conferred by Article X. They shall from time to time elect Members to the Corporation upon such terms and conditions as they shall have determined, not inconsistent with law or these bylaws.

X. (A) The Executive Committee is hereby designated to consist of not more than ten members, including the ex officio Members (Chairman of the Board of Trustees, President, Director and Treasurer); and six additional Trustees, two of whom shall be elected by the Board of Trustees each year, to serve for a three-year term.

(B) The Chairman of the Board of Trustees shall act as Chairman of the Executive Committee, and the President as Vice Chairman. A majority of the members of the Executive Committee shall constitute a quorum and the affirmative vote of a majority of those voting at any meeting at which a quorum is present shall constitute action on behalf of the Executive Committee. The Executive Committee shall meet at such times and places and upon such notice and appoint such sub-committees as the Committee shall determine.

(C) The Executive Committee shall have and may exercise all the powers of the Board during the intervals between meetings of the Board of Trustees except those powers specifically withheld from time to time by vote of the Board or by law.

(D) The Executive Committee shall keep appropriate minutes of its meetings and its action shall be reported to the Board of Trustees.

(E) The elected Members of the Executive Committee shall constitute as a standing "Committee for the Nomination of Officers", responsible for making nominations, at each Annual Meeting of the Corporation, and of the Board of Trustees, for candidates to fill each office as the respective terms of office expire (Chairman of the Board, President, Director, Treasurer, and Clerk).

XI. A majority of the Trustees then in office shall constitute a quorum. A lesser number than a quorum may adjourn any meeting from time to time without further notice.

XII. Any action required or permitted to be taken at any meeting of the Trustees or of the Executive Committee may be taken without a meeting if all the Trustees or members of the Executive Committee, as the case may be, consent to the action in writing and such written consents are filed with the records of meetings. Such a consent shall be treated for all purposes as a vote at a meeting.

XIII. The consent of every Trustee shall be necessary to dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be disposed of in such manner and upon such terms as shall be determined by the affirmative vote of two-thirds of the Board of Trustees then in office.

XIV. These bylaws may be amended by the affirmative vote of the Members at any meeting, provided that notice of the substance of the proposed amendment is stated in the notice of such meeting. As authorized by the Articles of Organization, the Trustees, by a majority of their number then in office, may also make, amend, or repeal these bylaws, in whole or in part, except with respect to (a) the provisions of these bylaws governing (i) the removal of Trustees and (ii) the amendment of these bylaws and (b) any provisions of these bylaws which by law, the Articles of Organization or these bylaws, requires action by the Members.

No later than the time of giving notice of the meeting of Members next following the making, amending or repealing by the Trustees of any bylaw, notice thereof stating the substance of such change shall be given to all Corporation Members entitled to vote on amending the bylaws.

Any bylaw adopted by the Trustees may be amended or repealed by the Members entitled to vote on amending the bylaws.

XV. The account of the Treasurer shall be audited annually by a certified public accountant.

V. REPORT OF THE DIRECTOR

To: The Trustees of the Marine Biological Laboratory

This report, prepared by a new Director installed on August 15, 1975, covers the period from that date until this writing, the middle of May, 1976. However, in the record of activities at the MBL there are no sharp beginnings or endings. One Director picks up where another leaves off and therefore what was initiated or discussed during the regime of one becomes the responsibility of the next to activate. Thus I have found myself accepting the reins from a great achiever among the Directors of MBL and one who is therefore difficult to succeed. That I have tried to follow Jim Ebert respectably will be evident in what follows, but to try is not necessarily to be successful. Our styles of administration are quite different and in inheriting the organization he had structured I felt obliged to make some minor changes.

Reorganization of administration

Gradually over the past few years the Laboratory has become more active as a year-round research and teaching center. The Boston University Marine Program under Arthur Humes, the Ecosystems Center under George Woodwell, the Laboratory of Sensory Physiology under E. F. MacNichol, Jr. and the Laboratory of Biophysics, NINCDS of the National Institutes of Health under William J. Adelman, Jr., not to mention several smaller and individual laboratories, have increased the number of investigators and research assistants in the winter laboratory to about 75 individuals and correspondingly increased the demand for supplies and services. In the month of January a four-week program of courses for undergraduates adds about 100 students and several instructors to the community. Partly to cope with this activity in his absence, Dr. Ebert had appointed E. F. MacNichol, Jr. as Assistant Director for Research Services and G. M. Woodwell as Assistant Director for Education. Further to manage and organize the financial aspects of the Institution he installed Charles Ossola as Assistant Director for Finance and Development late in 1975, with Edward G. Casey as Comptroller. Homer P. Smith remained as General Manager of the physical plant and secretary to the Executive Committee of the Trustees.

This division of responsibilities has worked fairly well, and this seems an appropriate place to acknowledge the fine cooperation I have received from these Administrative

Assistants during this period of adjustment. They, together with all the staff, have kept the Laboratory operating with only the part-time attention of a part-time Director.

Early in the year it became evident that communication between the several Assistant Directors and Department Heads was less than adequate and so we instituted a Staff Council which comprises the Assistant Directors and Department Heads. This has served well as a forum for the discussion of several management problems and as a device for exchange of information between different levels in the Administration. E. F. MacNichol, Jr. is serving as Chairman of the Council in this first year of its organization. The plan is to rotate the chairmanship among the Assistant Directors with the MBL Director attending *ex officio*.

It was this same increase in year-round activity together with the continuing problems of funding that convinced me as early in my tenure as December 1975 that the MBL needed leadership that only a full-time Director could provide. I therefore informed the Executive Committee at its Budget Meeting in December that I felt it should begin not later than the summer of 1976 a search for my successor, who would become resident Director, thus eliminating the necessity of the fragmented responsibility currently operating. This decision was made while I was still relatively objective about the MBL and was concurred in by Charles Ossola, who added that he thought the management unnecessarily expensive for the tasks involved. It was his further comment that a Director with a good Executive Secretary and the Department Heads could easily run the Laboratory, including its fund-raising program. The above admonition to the Executive Committee was repeated in February, 1976.

This seems an appropriate place to acknowledge the outstanding performance of Mr. Ossola as Assistant Director for Finance and Development and to report regretfully that he has decided to return to Washington. During the eight months that he was resident he brought order out of confusion in the financial end of MBL management.

A number of changes have been made in 1975–76 in top staff positions. Mr. Edward J. Bender died after twelve years of faithful service to the Laboratory. Mr. Frank A. Wildes left the Laboratory, having served as Comptroller for a little over eight years, and Mr. Jim A. Hancock, Manager of the Department of Research Services for over four years, left the MBL in the early spring of 1976.

The library

With each passing year our library increases in worth and significance and also in its deficit. Indeed we find ourselves the owners of a library we are less and less able to afford. It is a truly great heritage which long ago should have acquired an endowment of sufficient size to maintain the books and essential services if not to provide for new purchases. More and more the community of scientists in Woods Hole and environs depend on it as their source of information, and this community includes not only the resident group within MBL but also the staffs of the Woods Hole Oceanographic Institution, the N. O. A. A. Northeast Fisheries center and the U. S. Geological Survey. As the scholars in these latter institutions have come to appreciate the worth and importance of this facility their administrative officers have become more concerned with the operation of the Library and more willing to share the costs. Thus in this current year the Woods Hole Oceanographic Institution has increased its contribution by 50% to $30,000 for general operating costs, and the government agencies resident in Woods Hole have found a way to help with $10,000 each. This is very substantial assistance and is much appreciated. At best these represent temporary arrangements, however, and as such are without assurance of continuation. Thus we go out each season with hat in hand and return repeatedly with the desire to find a mechanism or mechanisms that will make the library a responsibility of the entire scientific community in both management and funding.

It is my pleasure to report that within the current year we have witnessed increasing interest on all sides in the establishment of a Woods Hole Scientific Library Association in which the MBL would share the Woods Hole Oceanographic Institution, on a nearly equal basis, the operation as well as the use of the library. This does not mean that ownership of the library is in any sense relinquished or that the MBL will have handed to another Institution a controlling interest in its future. It simply means that the Library will be made increasingly useful to the community, in return for which the community will share a larger portion of the responsibility—including the cost. I personally see no other practical or even better way to handle this problem. It seems likely, moreover, that the soundness of such a move will impress possible benefactors and make them more interested in funding the several improvements required. Certainly, in incorporating more of the special interests of oceanographic scientists, we improve the Library and greatly strengthen our fund-raising position.

To assist in designing a plan for the better operation of the library and the possible transition to a new management arrangement, Paul Fye (President of the Woods Hole Oceanographic Institution) has joined me in acquiring the consulting services of John Harrison, a highly recommended science librarian from Yale University. Mr. Harrison has in this period visited Woods Hole twice and has promised to give us his report and recommendations before summer, so that the Library Committee and the Corporation can consider them and build a plan hopefully for future community cooperation. As never before, this may be the Year of the Library.

In its physical aspects the Library has not changed greatly in the last year, but those changes that have been made are not without significance. A valuable service, known as the Xerox Room, has been moved for greater efficiency from the third to the second floor, into space immediately adjacent to the administrative offices of the library.

In addition, a room in the northwest corner of the third floor of Lillie has been fitted with shelves to accommodate the oldest numbers of some of the serials, such as the *Philosophical Transactions of the Royal Society of London.* This series is complete from 1667 to the present. Needless to say, such collections are fairly rare, seldom used, and therefore can better reside in a relatively dry, safe space where they will be available by key to qualified users. The shelf space thus released will be welcomed by the librarians to receive the ever-expanding serials in what may be referred to as the working library.

The Marine Resources Center

The inadequacies of the physical facilities of the present Supply Department have been recognized for several years. The annual reports from standing committees at the Laboratory have repeatedly commented on the obsolescence of the facility and have urged the Executive Committee to do something about it. Alternatively, the Committee on Buildings and Grounds has advised the Administration that the present building, erected in 1924, is gradually sliding into the Eel Pond.

This year the Administration decided to move and as a start it got the Executive Committee to budget funds for planning. An *ad hoc* committee of persons* qualified to judge the situation and advise was called into being and met in January 1976 with architects to outline the uses and design of such a new facility. The architects returned in February with a scaled model in which the proposed structure was placed with respect to its surroundings.

As envisioned by the *ad hoc* Committee and the Administration, the building will consist of three floors and approximately 35,000 square feet of floor area. The first, or ground, floor will provide a substantially expanded space for the current collecting and

* James Hanks, John Hughes, Robert Gunning, Fred Lang, John Rankin, John Ryther, John Valois, Shinya Inoué, Raymond Stephens, Cyrus Levinthal, George Streisinger, Charles Wheeler, Sears Crowell and Homer Smith (Chairman).

storage activities of the Department. This will include controlled systems for animal maintenance and an improved salt water system for uninterrupted flow during maintenance of filter beds. The second floor will be devoted to the culture of marine forms. This will involve the long-term maintenance of young and adult organisms and the controlled propagation of organisms to provide genetic stocks and certain species that are either scarce for the collector or difficult to obtain for reasons of accessibility or geography. The top, or third, floor will, according to present plans, be devoted to research into the genetics and pathology of marine organisms. Both of these interests will depend to greater or less extents on the aquaculture facility and experience developed on the second floor. The development of these plans and the programs involved will go forward through the summer months with the writing of applications for federal agency support.

This is clearly a large and interesting adventure that can be initiated only through the continuing support of individuals and foundations willing to invest in buildings for unique and largely unexplored objectives.

The teaching program

This continues to be one of the MBL's major activities and one in which it can take continuing pride. Students who enter the program seem in most instances to leave with an enduring commitment to biology.

During the year under review, the usual efforts have been made to obtain grant support for our courses. These have been successful in the case of the summer courses in Physiology (NIH), Embryology (NSF), the Microbial Ecology Training Program (Waksman Foundation for Microbiology) and Neurobiology (Grass Foundation and Merck Company Foundation). That leaves Ecology without support except through the Ecosystems Center; and Experimental Marine Botany and Experimental Invertebrate Zoology without support except through the MBL.

The cost of operating these courses, especially when grant support is not available, constitutes a serious drain on the limited resources of the MBL. We have therefore sought to take advantage of a new Research Initiation and Support Program (RIAS) announced this year by NSF. If our application is approved and funded beginning July 1, 1976, some assistance can be accorded the courses that are not otherwise supported. The same support will also initiate a new summer offering: a Seminar in Cell Biology designed especially for the student who has not had an opportunity to acquire training in this area.

The January Course offerings continue to increase in popularity, which may be taken as a mark of their success. Here also, in the hope of improving the instruction and facilities (equipment) for the students, we have sought to attract NSF support under their program: Comprehensive Assistance to Undergraduate Science Education (CAUSE). Should this be granted, we propose to include with the established January offerings in Ecology, Developmental Biology, Behavior and Neurobiology, a new course in Comparative Histology. This new offering will attempt to pull together for comparative study in lectures and laboratory the wealth of new information on cells and tissues from invertebrate as well as vertebrate animals and from electron as well as light microscopy.

An important segment of the educational program at MBL is now offered under the guidance of Arthur Humes and Boston University. Known as BUMP, it has assembled a faculty of five professors and enrolls a total of 30 graduate and 5 undergraduate students. The emphasis is on marine organisms, their zoology, physiology and ecology. Course credits and tuition are the business of Boston University as is also the awarding of degrees. The MBL houses the program for a consideration. As is ever the case, students are an essential and stimulating component of any community of scholars.

Thus it is that this community welcomes the BUMP program. Quite on their own this year, the students have organized a series of weekly, noontime seminars which have attracted a large attendance and have been generally applauded. Thus we find it easy to encourage Professor Humes in his efforts to expand and improve his program in the limited space available.

The summer courses, with their unexcelled traditions, continue to occupy center stage for many students of marine biology. They owe this position to the interest and devotion of course heads who come here every summer over stretches of several years to participate. Not least among these devoted instructors has been John Cebra, who has headed Physiology. Since he has only one year remaining in his tenure a search for his successor was instituted in 1975–76 and resulted in our acquiring the consent of K. E. Van Holde. He will apprentice during the summer of 1976 and take over his new responsibilities in 1977.

The Ecosystems Center

In his 1974 report Dr. Ebert reviewed the establishment of this year-round program and its generous funding by a number of foundations. The development of this Center at the MBL may be taken as an example to be followed as the Laboratory undertakes to assemble groups of investigators for year-round programs in other areas. As Dr. Ebert pointed out a year ago, one special attribute of the Center and the Laboratory is their ability to attract distinguished scientists from other institutions around the world. We should take advantage of this ability in the development of other year-round programs.

Within the period of this report Dr. George Woodwell has increased his staff by adding John Hobbie as Senior Scientist, and Jerry Melillo, Bruce Peterson and J. Tiwari as Assistant Scientists.

It became evident to the former Director in the summer of 1975 that a program as significant for the MBL as the Ecosystems Center should have a Visiting Committee that would monitor the activities of the Center and provide advice and guidance to the Executive Committee and the Director of the MBL. The committee appointed comprises E. O. Wilson (Harvard) as Chairman, Kenneth Mann (Dalhousie University), Howard Sanders (Woods Hole Oceanographic Institution), W. T. Edmondson (University of Washington) and R. H. Whittaker (Cornell). The Committee met in Woods Hole on January 22, 1976 and later provided a report which, it seems to me, in being the first on this unique program at the MBL, deserves to be recorded in these pages.

Report of the Ecosystems Center Visiting Committee

The Visiting Committee met at Woods Hole on January 22, 1976, to acquaint itself with the staff of the Ecosystems Center and to assess the progress made by this fledgling institution during its first year.

Our impressions were very favorable. Dr. George M. Woodwell's conception of the possible future role of the Ecosystems Center in international science appears to us to be entirely sound. He has chosen not simply to foster a set of separate programs in basic ecology, in the manner of traditional university departments, but rather to commit the center to several important projects in ecosystems studies which are well defined but nevertheless so large and eclectic as to require a special research center. To this end Dr. Woodwell has recruited a small scientific staff that includes several of the most talented specialists in the field. He himself has demonstrated the capacity to provide both the administrative skills and the unusual vision and knowledge required for successful ecosystems research.

As constituted at present, the Ecosystems Center is unique in this country. We foresee not only the possibility for success in the enterprises undertaken by the Center, including forecasts of the earth's carbon budget and of the effects of biotic impoverishment in several kinds of environments, but also the growth of other functions of potential importance to science. In particular, the members of the Center staff will be in an unusually strong position to advise other scientists concerning important research projects—they can function as "brokers" of ideas to aid scientists whose focus is necessarily more limited. They might also provide an early warning system, by being the first to spot potential environmental hazards in the form of new contaminants or ill effects due to the alteration of particular habitats. The great majority of applied scientists devote their time to hazards that have already become all too apparent; with its broader investigations, the Center might be fortunate enough to predict and forestall some of the problems.

Accordingly, it is important that the staff of the Ecosystems Center not become too isolated or specialized. At Woods Hole the opportunities abound for constant new learning and collaboration, yet we feel that no formal measures should be taken to secure this type of intellectual growth. The Center staff should instead be encouraged to make arrangements on an informal and personal basis as opportunities arise. For example, staff members might well serve on doctoral thesis committees in the MIT–WHOI marine sciences program, or at Harvard University—but only as they see fit.

The educational program undertaken by the Center Staff, particularly the January course in ecology at Woods Hole, is of exceptionally high quality. It seems certain not only to add to the future intellectual life of the Woods Hole community, but also to benefit the intellectual growth of the Staff members themselves. We hope it will be continued.

Although difficult to judge at this early stage, the present size of the Ecosystems Center scientific staff seems adequate to us. While future events may lead Dr. Woodwell to seek additional members, we see no reason why he should be urged to do so during the first several years. On the contrary, it is important for him not to become enmeshed in excessive administrative work. In ecosystems research, as in few other branches of science, it is crucial for the group director to have time in which to reflect on the future of his discipline while carrying on his own research, the two activities in which Dr. Woodwell has hitherto excelled.

Kenneth Mann
Howard Sanders
Edward O. Wilson, *Chairman*

Hirohito at the Laboratory

In early October the Laboratory, indeed all of Woods Hole, found itself in the spotlight. Boyce Rensberger's article on the front page of the *New York Times* for October 4 began: "For a few minutes today the Emperor of Japan departed from the ceremony that has marked his visit to the United States, and looking through the microscope at the Marine Biological Laboratory immersed himself in the watery world of primitive jellyfish-like creatures known as hydroids." It was not by accident that His Majesty chanced upon Woods Hole. Almost from the moment his state visit was announced the Emperor emphasized that visits to the Marine Biological Laboratory and the Woods Hole Oceanographic Institution were "musts." Hirohito is an accomplished marine biologist—a systematist—whose extensive publications in our library emphasize his interest in hydroids. However, Woods Hole has a special significance for His Majesty, a significance best expressed in Dr. Ebert's welcoming remarks: "Almost a century ago E. S. Morse, who had been a student of Agassiz, founder of this Nation's first seaside laboratory, journeyed to Japan where he became the first professor of zoology at the

University of Tokyo. He was followed soon thereafter by Charles Otis Whitman—then a young zoologist destined to become one of the pioneers in animal behavior and experimental embryology. In a few scant years Whitman returned to the United States where 88 years ago he became the first Director of the Marine Biological Laboratory. Thus Japanese and American marine biology are fed by a common wellspring, nourished by Morse's enthusiasm for natural history and systematics and sparked by Whitman's creativity, drive and remarkable capacity for organization. It is no small wonder, then, that Japanese and American scientists have always stood—in fact, stand together today—at the cutting edge of research in marine biology.

"Nor is it an accident that the roots of Japanese-American cooperation run deep at the Marine Biological Laboratory, indeed throughout the scientific community of Woods Hole. There is a rich tradition of Japanese scientists who have journeyed to Woods Hole to work in our unique 'family' of scientific institutions. They have come as distinguished investigators, devoted teachers and students; they have forged new trails participating in notable discovery and serving as models for our students."

At His Majesty's request formal ceremonies were brief. The Emperor expressed his interest in "getting on with the science." It is clear that his stay in Woods Hole was one of the highlights of the Emperor's visit to the United States. So many members of the Laboratory's staff participated in making the day an outstanding success that it is difficult to single out even a few for special mention. It should be recorded, however, that the scientific party meeting with His Majesty included Sears Crowell, Shinya Inoué, Patricia Morse and Marie Abbott, with Hidemi Sato acting as an interpreter.

1. Memorials

ARNOLD LAZAROW

By Berta Scharrer

On June 25, 1975, the scientific community was saddened by the untimely death of Dr. Arnold Lazarow, distinguished investigator and inspired educator. My long friendship with him goes back to his student days and I followed his ascent with great admiration. It is hard to accept the fact that he is no longer with us.

Born in Detroit on August 3, 1916, Arnold received his academic education at the University of Chicago. Always a prodigious and highly motivated worker, he combined his medical training with research, specializing in anatomy and biochemistry, and in 1941 was granted both an M.D. and a Ph.D. degree. After his internship and a brief postdoctoral period with Dr. Gordon H. Scott, he participated in a war research project at the University of Southern California. In 1943 he joined the faculty of Western Reserve University, Cleveland, Ohio, where for eleven years he remained a very productive member of the Department of Anatomy, then headed by Dr. Normand L. Hoerr.

From 1954–1975 Arnold Lazarow was professor and head of the Department of Anatomy at the University of Minnesota Medical School. During this period he brought great distinction to this department and to the discipline of Anatomy, by gaining an international reputation as a brilliant scientist and by his dynamic contributions to medical education and to the training of research investigators.

The more than 200 publications of Arnold Lazarow span a broad range of topics and reveal his interest in cytochemical and quantitative methodology, including computer technology. Under the leadership of his much admired mentor, Dr. Robert R. Bensley, the young predoctoral fellow pioneered in the fractionation and functional analysis of subcellular components (mitochondria, particulate glycogen).

The central theme of his investigative efforts, and the one that held the greatest fascination for him throughout his life, was the problem of diabetes. The success of his multifaceted approach to the solution of this basic and clinically so important problem bespeaks his broad biomedical background, his expertise in instrumentation, his great drive, and his capacity to attract and inspire capable collaborators.

The principal accomplishments of Dr. Lazarow and his group in this area center on first, the elucidation of the selectively destructive effects of diabetogenic agents (especially alloxan) on the insulin-producing beta cells of the pancreatic islet; secondly, the analysis of control mechanisms responsible for insulin synthesis, storage, and release; thirdly, the development of a diagnostically important immunoassay method for the determination of insulin levels in the plasma; and fourthly, the search for potential curative uses of transplants of pancreatic islet cells derived from organ cultures. For this distinguished work Dr. Lazarow received the Banting Medal in 1973, the highest honor of the American Diabetes Association.

The original impetus for Arnold's long and enthusiastic association with the Marine Biological Laboratory was one of nature's handy experiments, *i.e.*, the spatial separation of insulin-secreting cells from the rest of the pancreatic tissue in teleost fishes. This ideally suited material permitted him and his team to carry out numerous important studies during many busy summers starting in 1944. But perhaps the ready availability of this material *per se* would not have been sufficient to bring Arnold and his family back to Woods Hole year after year as summer residents. He greatly enjoyed the intellectual ferment, the informality and ease of daily scientific exchange, and the opportunity of working in a relaxed atmosphere in lieu of a "vacation". In 1960 he became a trustee of the M.B.L., and he also received the honor of being selected as one of its "Friday Evening Lecturers."

Dr. Lazarow was a forceful proponent of the value of well-planned basic research and of the need for a solid morphological foundation in all physiological and biochemical experimentation. He also demonstrated that a successful research career leaves room for other academic pursuits.

He was an inspiring and highly respected teacher with a genuine understanding for the needs of young people. His students and his colleagues were very loyal to him; they appreciated his keen mind, his youthful enthusiasm and scientific curiosity, and his cheerful, open nature. He had a natural courtesy and remained calm in difficult situations. He was also a good administrator. There were many demands on him as a lecturer and consultant, both here and abroad. He also served on several important national committees and editorial boards, especially that of *Diabetes*.

Without doubt, much of the energy for all of these endeavors stemmed from the support by Arnold's family. He was justifiably proud of his charming and devoted wife Jane, and his sons Paul and Normand, both of whom followed their father in selecting biomedical careers. Jane was not only his closest companion and a splendid hostess to a wide circle of friends and colleagues; she also became very active and proficient in one of her husband's ancillary efforts, the development and use of advanced methods for the storage and retrieval of scientific information.

Arnold Lazarow will be warmly remembered not only for his lasting accomplishments but for his fine human qualities, his generosity, integrity and modesty, his gentle concern for others, his gift of friendship.

2. The Staff

EMBRYOLOGY

I. Instructors

David Epel, University of California, San Diego, co-director
Tom Humphreys, University of Hawaii, co-director

Merton Bernfield, Stanford University School of Medicine
Jean-Paul Revel, California Institute of Technology

II. Consultants

Saul Roseman, The Johns Hopkins University
Melvin Spiegel, Dartmouth College
Theodore Steck, University of Chicago

III. Assistants

Roger Duncan, University of Hawaii
James Johnson, University of California, San Diego

IV. Lectures

S. J. Singer	Molecular organization of membranes
Jean–Paul Revel	The anatomy of cell surfaces
David Epel	Ions and surface proteins and activation of the egg at fertilization
Victor Vacquier	Cortical granules, secretory vesicles and the mechanism of exocytosis
Tom Humphreys	Biochemical studies on the aggregation of marine sponge cells
J. Hermolin	Aggregation promoting materials from chick embryo cells
Steve Rosen	Carbohydrate binding proteins may mediate intercellular adhesion in slime molds
Melvin Spiegel	Specific cell adhesion in embryonic sea urchin cells
Jean–Paul Revel	Cell junctions during the interactions between cells
T. Steck	Membrane molecular architecture
T. Steck	Organization of the red cell membrane
Kayo Okazaki	Differentiation of isolated sea urchin embryo micromeres *in vitro*
V. Hascall	Aggregation of cartilage proteoglycans
Merton Bernfield	Extracellular matrices in morphogensis
Norm Wessels	Environmental effects on nerve growth
Saul Roseman	Metabolism of cell surface complex carbohydrates and their potential role in intercellular adhesion. Part I
Saul Roseman	Metabolism of cell surface complex carbohydrates and their potential role in intercellular adhesion. Part II
Saul Roseman	Serendipity and sialic acid
Lewis Tilney	Filamentous cellular organelles and the acrosome reaction
Richard Hynes	Surface proteins of normal and transformed cells
Daniel Rifkin	Proteases and malignant transformation
David McClay	Specificity of aggregation of hybrid sea urchin cells
Bryan Toole	Morphogenetic role of extracellular glycosamino-glycans
Struther Arnott	How polysaccharides stick together

EXPERIMENTAL INVERTEBRATE ZOOLOGY

I. Instructors

Michael J. Greenberg, Florida State University, director of course
Stephen H. Bishop, Baylor College of Medicine, Houston
John H. Crowe, University of California, Davis

Ann E. Kammer, Kansas State University
Larry C. Oglesby, College of William and Mary
Rosevelt L. Pardy, University of California, Irvine
Sidney K. Pierce, Jr., University of Maryland
Thomas J. M. Schopf, University of Chicago
Kenneth B. Storey, Duke University

II. Consultants

F. A. Brown, Jr., Northwestern University
C. Ladd Prosser, University of Illinois, Urbana
Alfred C. Redfield, Woods Hole
W. D. Russell–Hunter, Syracuse University
James Case, University of California, Santa Barbara
Robert K. Josephson, University of California, Irvine

III. Assistants

Rebecca B. Price, Florida State University
Charlene Reed, Florida State University
Wendy Wiltse, University of Massachusetts

IV. Secretary

Mrs. Christine Pierce, Marine Biological Laboratory

V. Special Lecturers

Frank A. Belamarich, Boston University
John O. Corliss, University of Maryland
Alan Gelperin, Princeton University
Stephen J. Gould, Harvard University
John L. Roberts, University of Massachusetts
Charlotte P. Mangum, College of William and Mary
Toni Steinacher, Albert Einstein College of Medicine

VI. Lectures

M. J. Greenberg	A few words from the course director
L. C. Oglesby	An introduction to salinity tolerance and water balance
T. J. M. Schopf	The Cape Cod environment, past and present
S. K. Pierce	The problem of osmotic regulation in osmoconforming animals and a fast look at membrane structure
S. K. Pierce	Cell volume regulation: the free amino acid story
L. C. Oglesby	Extracellular osmotic regulation: ions and active transport
L. C. Oglesby	Energetics of ion transport
S. K. Pierce	Excretory systems and water balance
S. H. Bishop	Nitrogen nutritional requirements and intermediary metabolism
S. H. Bishop	Ammonia forming mechanisms
S. H. Bishop	Ammonia detoxification mechanisms: uric acid, urea, amino acid
J. O. Corliss	Nuclear characteristics and phylogeny in the ciliates
S. H. Bishop	Nitrogen elimination
C. P. Mangum	The respiratory pigments

C. P. Mangum	On the function of the primitive red cell
J. L. Roberts	Gaseous exchange across the gills
A. E. Kammer	Introduction to neural control mechanisms
A. E. Kammer	Neural control of ventilation in invertebrates
J. L. Roberts	Respiratory accommodation for swimming in fish
M. J. Greenberg	Circulation: although variation is large, some generalizations about structure and function emerge
M. J. Greenberg	Hearts and other pumps: operation and regulation
M. J. Greenberg	How much do we know about adaptation of the circulation to the environment in invertebrates
K. B. Storey	Invertebrate anaerobiosis: basic biochemical mechanisms
K. B. Storey	Animals in oxygen: biochemical strategies
K. B. Storey	Biochemical adaptations to temperature changes: enzymes and their environments
F. A. Belamarich	Hemostasis in the invertebrates
J. H. Crowe	The induction of anhydrobiosis in invertebrates: life without water
J. H. Crowe	Anhydrobiosis: a problem in defining "life"; cryobiosis and chilling injuries
J. H. Crowe	Life in xeric environments: behavioral, physiological, and ecological adaptations to deserts
J. H. Crowe	Life in xeric environments: absorption of water-vapor from subsaturated air by desert insects
A. E. Kammer	Temperature and invertebrates
A. E. Kammer	Regulation of body temperature
T. Steinacher	Auxillary hearts and the control of blood pressure in decapod Crustacea
R. L. Pardy	Feeding and digestion in marine invertebrates
R. L. Pardy	Chemical control of feeding and amino acid uptake
R. L. Pardy	Introduction to symbiosis
R. L. Pardy	Algal and chloroplast endosymbiosis
M. J. Greenberg	There is more to feeding and digestion in bivalved molluscs than anyone ever supposed
S. Gould	Stochastic models of phylogeny
A. Gelperin	Neuroethological studies of feeding and learning in a gastropod mollusk
T. J. M. Schopf	Rates of evolution
T. J. M. Schopf	Crises in the history of life: marine extinctions of the Permian
T. J. M. Schopf	Models of speciation: ergonomics of specialization
S. H. Bishop	Novel glycoproteins from marine invertebrates: phosphonoproteins

EXPERIMENTAL MARINE BOTANY

(Comparative Biology and Biochemistry of Algae)

I. Instructors

Jerome A. Schiff, Brandeis University, director of course
Harvard Lyman, State University of New York at Stonybrook
James R. Sears, Southeastern Massachusetts University

II. Consultants

Robert L. Guillard, Woods Hole Oceanographic Institution
David Mauzerall, The Rockefeller University

III. Assistants

Phillip Clemons, Southeastern Massachusetts University
Karen Lahey, State University of New York at Stonybrook

IV. Lectures

Jerome A. Schiff	Chemical phase of evolution: biogeochemistry
Jerome A. Schiff	The appearance of oxygen
Jerome A. Schiff	Evolution of procaryotes
Jerome A. Schiff	Evolution of eucaryotes and organelles
F. J. R. Taylor	Theories for the origin of eucaryotes
Jerome A. Schiff	Evolution of life cycles
Jerome A. Schiff	Nutritional cycles
Jerome A. Schiff	Metabolism of nitrogen and sulfur
Mary M. Allen	Ecology of blue green algae
Mary M. Allen	Physiology of blue green algae
Anthony E. Walsby	The heterocyst and nitrogen fixation
Anthony E. Walsby	Ecology of planktonic blue green algae
Anthony E. Walsby	Buoyancy mechanisms in blue green and other algae
Moshe Shilo	Blue green algal viruses (Cyanophages)
Harvard Lyman	Algal symbioses
Samuel I. Beale	Heme and chlorophyll biosynthesis: early steps
Robert F. Troxler	Heme and chlorophyll biosynthesis: later steps
Robert F. Troxler	Biosynthesis of open chain tetrapyrroles
Joseph S. Ramus	Chromatic adaptation
James R. Sears	Vertical distribution and productivity
David Mauzerall	Photochemistry of photosynthesis
Jane Gibson	Photosynthetic electron transport and phosphorylation
Martin Gibbs	Photoreduction of CO_2 and H_2
Gary Kochert	Sexual differentiation in algae
Gary Kochert	Algal gamones
Gary Kochert	Establishment of polarity in algae
Ray Stevens	Algal microtubules
Leland Edmunds Jr.	Algal clocks and rhythms

V. Laboratory Lectures

James R. Sears	Chlorophyta I
James R. Sears	Chlorophyta II
F. J. R. Taylor	Dinoflagellates
James R. Sears	Chlorophyta III
Harvard Lyman	Euglenophyta
Harvard Lyman	Pigment extraction and identification
Harvard Lyman	Extraction and estimation of cellular components
James R. Sears	Phaeophyta I
James R. Sears	Phaeophyta II
James R. Sears	Phaeophyta III
Ian Morris	Biochemical adaptation in phytoplankton

Harvard Lyman	Algal toxins
Frederick Kazama	Marine fungi
Harvard Lyman	Calcification
Harvard Lyman	Thermophilic algae
James R. Sears	Rhodophyta I
James R. Sears	Rhodophyta II
James R. Sears	Rhodophyta III
James R. Sears and Harvard Lyman	Techniques for measuring primary production
James R. Sears and Harvard Lyman	Discussion of physiological ecology
Robert L. Guillard	Chrysophyta: systematics and ecology, phytoplankton ecology
James R. Sears	Xanthophyta and bacillariophyta

ECOLOGY

I. Instructors

Frederick E. Smith, Harvard University, director of course
George M. Woodwell, Marine Biological Laboratory, associate director of course
Daniel B. Botkin, Marine Biological Laboratory
Charles A. S. Hall, Marine Biological Laboratory

II. Assistant

Thomas K. Duncan, Boston University

III. Special Lecturers

Aimlee D. Laderman, Ramapo College of New Jersey
Thomas Lovejoy, World Wildlife Fund, Washington, D. C.
Ramon Margalef, University of Barcelona, Spain
Jane Menge, Harvard University
Bruce Menge, University of Massachusetts, Boston
Richard Osman, University of Chicago
Robert T. Paine, University of Washington
Howard Sanders, Woods Hole Oceanographic Institution
Lawrence B. Slobodkin, State University of New York at Stony Brook
Otto Solbrig, Harvard University
Donald Tilton, University of Minnesota
Robert H. Whittaker, Cornell University
Ivan Valiela, Boston University

IV. Lectures

F. E. Smith	General ecology, evolutionary ecology, and systems ecology
F. E. Smith	Ecosystem concepts
F. E. Smith	Problems in the study of marine systems
I. Valiela	Salt marsh ecology
T. K. Duncan	Estuarine ecology
H. Sanders	Marine benthic ecology I
H. Sanders	Marine benthic ecology II
R. Margalef	Extra energy in ecosystems
F. E. Smith	The international biological program: U. S. contribution

O. Solbrig	Convergent evolution in ecosystems
F. E. Smith	The planets as environmental systems
F. E. Smith	More on earth systems
D. Tilton	The growth and nutrition of the larch, *Larix laricina*
R. Osman	The species equilibrium theory: rocks as islands
R. Osman	The role of disturbance in a marine epifaunal community
R. T. Paine	Predation, body size, and community structure
R. T. Paine	Spatial and temporal pattern in a rocky intertidal community
L. B. Slobodkin	The peculiar evolutionary strategy of man
L. B. Slobodkin	Evolutionary infrastructure of ecosystem analysis
F. E. Smith	Feeding links and food chains in ecosystems
G. M. Woodwell	The vegetation of the earth, I
G. M. Woodwell	The vegetation of the earth, II
R. H. Whittaker	On the structure of communities, I
R. H. Whittaker	On the structure of communities, II
D. B. Botkin	Succession in terrestrial ecosystems
D. B. Botkin	Isle Royale: the structure and function of a terrestrial ecosystem
D. B. Botkin	An African contrast
G. M. Woodwell	An estuarine contrast
G. M. Woodwell	A case history study of biotic impoverishment
G. M. Woodwell	Toxic substances and ecological cycles
F. E. Smith	Population regulation in ecosystems and in models
F. E. Smith	Stability in ecosystems and in models
R. H. Whittaker	Indirect ordination and evolution of communities, I
R. H. Whittaker	Indirect ordination and evolution of communities, II
F. E. Smith	Emergent properties of ecosystems and of models
T. Lovejoy	Tropical rain forests: structure and diversity
J. Menge	Organization of a New England rocky intertidal system: I. Effects of herbivory, competition, and wave action on plants
B. Menge	Organization of a New England rocky intertidal system: II. Effects of predation, competition, and wave shock on primary space occupancy
F. E. Smith	Models in ecological research vs. ecosystems in modeling research
A. D. Laderman	Cedar swamp ecology
F. E. Smith	Comparisons of marine, benthic, intertidal, estuarine, and terrestrial systems
F. E. Smith	Relationships between the quality of life and population density: actual, optimal, and maximal densities as a function of human progress

NEUROBIOLOGY

I. Instructors

Edward A. Kravitz, Harvard Medical School, co-director of course
Antony O. W. Stretton, Wisconsin University, co-director of course
Philippa Claude, Harvard Medical School
Gerald D. Fischbach, Harvard Medical School
Edwin J. Furshpan, Harvard Medical School

ZACH W. HALL, Harvard Medical School
JOHN HEUSER, University of California, San Francisco
PAUL O'LAGUE, Harvard Medical School
DAVID D. POTTER, Harvard Medical School
THOMAS S. REESE, National Institutes of Health

II. ASSISTANT

R. SIEGEL, Harvard Medical School

III. SPECIAL LECTURERS

D. YOSHIKAMI, Harvard Medical School
S. W. KUFFLER, Harvard Medical School
H. C. HARTZELL, Harvard Medical School
M. E. KRIEBEL, State University of New York, Upstate Medical Center
R. LLINAS, University of Iowa
T. HUMPHREYS, University of Hawaii
G. D. PAPPAS, Albert Einstein College of Medicine
M. V. L. BENNETT, Albert Einstein College of Medicine
S. M. HIGHSTEIN, Albert Einstein College of Medicine
R. J. LASEK, Case Western Reserve University
J. E. DOWLING, Harvard University
J. H. SCHWARTZ, Columbia University
P. PATTERSON, Harvard Medical School
J. G. NICHOLLS, Stanford University Medical School
D. KENNEDY, Stanford University
E. KANDEL, Columbia University
S. WARD, Harvard Medical School
C. LEVINTHAL, Columbia University
P. RAKIC, Children's Hospital Medical Center
T. N. WIESEL, Harvard Medical School
S. LEVAY, Harvard Medical School
M. STRYKER, Harvard Medical School
C. SHATZ, Harvard Medical School

IV. STAFF ASSOCIATES

J. M. LAFRATTA, Harvard Medical School
D. FARB, Harvard Medical School
B. HALL, National Institutes of Health
J. LAUER, Harvard Medical School
P. D. EVANS, Harvard Medical School
W. R. WOODWARD, Harvard Medical School
B. A. BATTELLE, Harvard Medical School
J. P. WALROND, University of Wisconsin

V. LECTURES

G. D. FISCHBACH	Aspects of myogenesis
G. D. FISCHBACH	Aspects of neurogenesis
G. D. FISCHBACH	Development of electrical and chemical excitability
G. D. FISCHBACH	Synapse formation
Z. W. HALL	Regulation of ACh receptors

J. Heuser	The nerve cell
T. S. Reese	Other neural cells
J. Heuser	The synapse; secretion I
J. Heuser	The synapse; secretion II
T. S. Reese	The synapse; reception
P. Claude	The synapse; development
P. Claude	Axoplasmic transport
Z. W. Hall	Properties of the acetylcholine receptor
Z. W. Hall	Junctional and extrajunctional acetylcholine receptors
A. O. W. Stretton	Acetylcholine
A. O. W. Stretton	Catecholamines
E. A. Kravitz	GABA

PHYSIOLOGY

I. Instructors

John J. Cebra, The Johns Hopkins University, director of course
Thomas Bachi, University of Zurich, Switzerland
Dennis Barrett, University of Denver
P. C. Huang, The Johns Hopkins University
Ru-chih C. Huang, The Johns Hopkins University
Paul A. Klein, University of Florida, College of Medicine
Thomas Pollard, Harvard Medical School
Dennis Powers, The Johns Hopkins University
Robert A. Prendergast, The Johns Hopkins University School of Medicine
Gerald Weissmann, New York University School of Medicine

II. Consultants

Gary Ackers, University of Virginia
Allen Edmundson, Argonne National Laboratory
Daniel Goodenough, Harvard Medical School
Raymond Hamers, Free University of Belgium
Jean Lindenmann, University of Zurich, Switzerland
Carol Reinisch, Sidney Farber Cancer Center
Matthew Scharff, Albert Einstein College of Medicine

III. Assistants

Martha Barrett, University of Colorado, Denver
Ellen Kraig, Brandeis University

IV. Special Lecturers

Thomas Baldwin, Harvard University
Gary Borisy, University of Wisconsin
Joseph Ilan, Case Western Reserve Medical School
Shinya Inoué, University of Pennsylvania
Richard Linck, Harvard Medical School
Richard Laursen, Boston University
Clarke Millette, Harvard Medical School
Manfred Nahmmacher, Leitz Company
John Robbins, Bureau of Biological Standards
H. S. Rosenkrantz, Columbia University College of Physicians and Surgeons

Raymond Stephens, Brandeis University and Marine Biological Laboratory
Thomas Stossel, Children's Hospital, Boston
Andrew Szent-Gyorgyi, Brandeis University
Leon Weiss, The Johns Hopkins University School of Medicine

V. Staff Associates

Paul Black, The Johns Hopkins University
Tucker Collins, University of Rochester Medical School
Randy Emmons, Washington and Lee University
Rebecca Emmons, University of Virginia Medical School
Alex Evers, New York University Medical School
Douglass Forbes, University of Oregon
Keigi Fujiwara, Harvard Medical School
William Marzluff, University of South Florida
Sharon Reed, Harvard Medical School
Charles Rice, California Institute of Technology
Nancy Weigel, The Johns Hopkins University

VI. Lectures

D. Goodenough	The structure of biomembranes: historical perspectives
D. Goodenough	The structure of biomembranes: current theories
D. Goodenough	Structure and function of some intercellular junctions
G. Weissmann	Liposomes as model membranes
J. J. Cebra	T- and B-lymphocytes; differentiation, final maturation and interaction
R. Emmons	Approaches to selective labeling of membrane proteins and their isolation
R. Prendergast	Cellular immune phenomena *in vitro*; activities of lymphocytes and lymphokines
J. J. Cebra	Detailed structure of antibodies relevant to the function of their component domains
J. B. Robbins	Principles for the induction of protective immunity
R. Prendergast	Cellular immune phenomena *in vitro*
C. Reinisch	Tumor immunity
L. Weiss	The architecture of lymphoid tissues and the circulation of their cells
G. Weissmann	Lysosomes and inflammation
G. Weissmann	Cyclic nucleotide control of leukocyte function
R. C. Huang	Eukaryotic transcription, I
R. C. Huang	Eukaryotic transcription, II
D. Barrett	Transcriptional control of development
P. C. Huang	The contribution of Fred Sanger to the knowledge of nucleic acid sequencing: the wisdom behind the methods which opened up the field
P. C. Huang	Knowledge of RNA's as derived from their primary nucleotide sequences—the findings after the breakthrough
P. C. Huang	Information on the primary nucleotide sequences of DNA—repetitiveness, symmetry and specificity
D. Powers	The structure, function and molecular ecology of fish hemoglobins
D. Powers	Isozymes and population studies
A. Edmundson	Three-dimensional structure of proteins
A. Edmundson	Divergent evolution of immunoglobulin domains

G. Ackers	Thermodynamics of molecular interactions applied to macromolecules
G. Ackers	Inter-subunit interactions in hemoglobins
T. Pollard, S. Inoue	Introduction to motile systems mitosis
G. Borisy	Microtubule assembly
T. Pollard	Force generation in muscle
A. Szent-Gyorgyi	Control of muscle contraction
T. Pollard	Cytoplasmic contractile proteins
T. Pollard	Molecular basis of cell motility
T. Bachi	Cell membranes and viral infections
T. Bachi	Virus—erythrocyte interactions
P. Klein	Immunobiology of virus-cell membrane interactions, I
P. Klein	Immunobiology of virus-cell membrane interactions, II
P. Klein	Immunobiology of virus-cell membrane interactions, III
R. C. Huang	Specific genes and gene products: *in vitro* translating systems
M. D. Scharff	Biosynthesis, assembly and secretion of proteins (immunoglobulins)
M. D. Scharff	Use of mutant cells to analyze protein biosynthesis, assembly and secretion
M. D. Scharff	Mammalian cell hybridization to study the expression of differentiated functions
H. S. Rosenkranz	Mutagenesis and environmental cancer
W. Marzluff	*In vitro* approaches to transcription control in eukaryotic cells
C. Millette	Surface components of mammalian spermatozoa
J. Ilan	Involvement of membrane in direct channeling of amino acids to the site of protein synthesis
R. Linck	Cilia and flagella, I
R. Stephens	Cilia and flagella, II
T. Stossel	Cellular contractile proteins and phagocytosis
J. Lindenmann	Immunopotentiation by enveloped viruses
J. Lindenmann	Antibodies against recognition structures for alloantigens
J. Lindenmann	T-cell receptors for alloantigens
D. Powers	Strategy of protein purification
G. Ackers	Physical characterization of proteins, I
G. Ackers	Physical characterization of proteins, II
J. J. Cebra	Chemical modification of proteins
T. Baldwin	Chemical characterization of proteins
R. Laursen	Sequential degradation of proteins
A. Edmundson	Crystallography of proteins: three dimensional structure
T. Pollard	Optical diffraction of electron micrographs
M. Nahmmacher	Qualitative and quantitative aspects of fluorescence microscopy

JANUARY COURSES 1975

BEHAVIOR

(*Offered Jointly by Boston University Marine Program and the Marine Biological Laboratory*)

I. Instructors and Special Lecturers

Donald Griffin, The Rockefeller University
Charles Walcott, State University of New York at Stony Brook

Carl Rettenmeyer, University of Connecticut
Alastair Stuart, University of Massachusetts, Amherst
George Michel, Boston University
Jeremy Hatch, University of Massachusetts, Boston
Celia Moore, University of Massachusetts, Boston
Loris Roth, U. S. Army Natick Laboratories
Jelle Atema, Woods Hole Oceanographic Institution
Adrianus Kalmijn, Woods Hole Oceanographic Institution
J. Stanley Cobb, University of Rhode Island
Robert L. Jeanne, Boston University, director of course

II. Lectures and Seminars

R. L. Jeanne	Introduction to the course
J. Atema	Sensory physiology and behavior
J. Atema	Structure and function of chemo- and mechanoreception
J. Atema	Evolution of chemoreception
A. Kalmijn	Electroreception in object and prey detection
A. Kalmijn	Orientation and navigation in electric and magnetic fields
J. S. Cobb	Instinct and motivation
J. S. Cobb	Burrowing behavior: an introduction to sequential analysis
J. S. Cobb	Dominance relations and molting patterns in lobsters
G. Michel	The role of learning in behavior
C. Moore	Constraints on learning
C. Moore	Hormones and experience in ring dove reproduction
R. L. Jeanne	Endogenous vs. exogenous control of biological rhythms
R. L. Jeanne	Introduction to orientation
D. Griffin	Natural migrations of wild birds
D. Griffin	Echolocation in bats and other animals
C. Walcott	Homing in pigeons
C. Walcott, D. Griffin	Evidence for sensitivity to the earth's magnetic field
C. Walcott, D. Griffin	Information transfer in bee dances
J. Atema	Introduction to communication
R. L. Jeanne	Information theory and the analysis of communication
L. Roth	Courtship and mating behavior in cockroaches
L. Roth	Oviposition behavior: its evolution and control
R. L. Jeanne	Introduction to sociobiology
J. Hatch	Social groupings in vertebrates in relation to resource distribution
J. Hatch	Piracy in laughing gulls: an example of the selfish group
G. Michel	Primate socialization
G. Michel	Kidnapping and care-taking in primates
R. L. Jeanne	Social behavior in the Hymenoptera
R. L. Jeanne	The genetical theory of sociality in the Hymenoptera
R. L. Jeanne	The adaptiveness of nesting behavior in the social wasps
A. Stuart	Social behavior in the termites
R. L. Jeanne	Organization of insect colonies
C. Rettenmeyer	Ant/plant interactions
C. Rettenmeyer	Ants as predators, with emphasis on army ants
C. Rettenmeyer	Symbioses between social insects and their guests
R. L. Jeanne	Evolution of behavior

DEVELOPMENTAL BIOLOGY

I. Instructors

Annette W. Coleman, Brown University
John R. Coleman, Brown University
Louis E. DeLanney, Ithaca College, director of course
Noel de Terra, The Institute for Cancer Research
James D. Ebert, Carnegie Institution of Washington and Marine Biological Laboratory
Joanne E. Fortune, Cornell University
Walter S. Vincent, University of Delaware
J. Richard Whittaker, Wistar Institute, associate director of course

II. Special Lecturers

Eugene Bell, Massachusetts Institute of Technology
Frances P. Bowles, Marine Biological Laboratory
Chandler Fulton, Brandeis University
Susan Gerbi, Brown University
Richard Goss, Brown University
Elizabeth Hay, Harvard Medical School
Arthur Humes, Boston University Marine Program at Marine Biological Laboratory
Fotis Kafatos, Harvard University
Raymond Stephens, Brandeis University and Marine Biological Laboratory
Albert Szent-Gyorgyi, Marine Biological Laboratory
J. P. Trinkaus, Yale University
E. Zuckerkandl, Centre de Recherches de Biochimie Macromoleculaire, Montpellier, France

III. Lectures

James D. Ebert	Introduction to MBL
James D. Ebert	Interacting systems in development
James D. Ebert	Perspectives in molecular genetics and development
L. E. DeLanney	Order and diversity in the living world: an introduction
J. R. Whittaker	Sponges and Coelenterates
J. R. Whittaker	Spiralians
Hans Laufer	Arthropods
J. R. Whittaker	Ascidians
J. D. Ebert	Ions and membranes
N. de Terra	A world in a grain of sand—what Ciliates can contribute to the study of development
J. R. Coleman	Structure and function of the eukaryote genome
J. R. Whittaker	Cytoplasmic information in development
L. E. DeLanney	Problems of vertebrate development
W. S. Vincent	The egg as a lesson in cell biology
W. S. Vincent	Ovary vs. testis: genetics vs. environment?
W. S. Vincent	Oocyte development: I. Cell organelles; II. The germinal vesicle nucleus; III. The nucleus, continued
W. S. Vincent	Rate control of gene expression: the ribosomal gene model
E. Zuckerkandl	The appearance of new protein sequence and function during evolution

Chandler Fulton	Flagellar tubulin synthesis and body shape changes in cell differentiation of *Naegleria*
Eugene Bell	Control systems in differentiation: the duality of the genome
Arthur Humes	Post-embryonic development of Copepoda
Francis P. Bowles	Lobsters and lobstermen
J. E. Fortune	Hormonal activity in development
J. R. Coleman	Models of tissue differentiation
A. W. Coleman	Plant development
J. R. Whittaker	Integration of problems in developmental biology

IV. Evening Seminars

J. R. Whittaker	Finding information about developmental biology and developmental biologists
Raymond Stephens	Ciliogenesis
Elizabeth Hay	Tissue interaction in the developing cornea
Susan Gerbi	Evolution of nucleotide sequences
Fotis Kafatos	Specific protein synthesis
Richard Goss	Regeneration and organ growth regulation
J. P. Trinkaus	Epiboly and cell movements in *Fundulus* embryos
Albert Szent-Gyorgyi	A submolecular approach to cancer

V. Student-Originated Seminars

William Bates	Reaggregation of mammalian brain cells *in vitro*
Norman Bensky	Masked messenger RNA in developing sea urchin embryos
Mark Kowalski	Determination of the site of mutant gene action—or befriending your local Gynandromorph

NEUROBIOLOGY

(*Offered jointly by Boston University Marine Program and the Marine Biological Laboratory*)

I. Instructors

Alan Fein, Marine Biological Laboratory
Edward F. MacNichol, Jr., Marine Biological Laboratory
Fred Lang, Boston University Marine Program, director of course

II. Guest Lecturers

Jack Byrne, New York University
Cyrus Levinthal, Columbia University
Edward Kravitz, Harvard Medical School
Gerald Fischbach, Harvard Medical School
John Dowling, Harvard University
Kenneth Roeder, Tufts University

III. Lectures by Instructors

Elementary electricity
Electrophysiological techniques
Neuron doctrine

Diffusion potentials
Resting potentials
Action potentials: extracellular and intracellular, cable theory
Action potentials: local circuit theory
Action potentials: voltage clamp
Action potentials: pharmacology
Action potentials: other considerations
Synaptic transmission: historical perspective, morphology and ultrastructure
Synaptic transmission: transmitter release, frog neuromuscular junction
Synaptic transmission: inhibition; crustacean neuromuscular junction
Synaptic transmission: recent advances
Principles of sensory physiology: morphology and physiology
Principles of sensory physiology: coding
Photoreception: invertebrate vision I
Photoreception: vertebrate vision II
Mechanoreception: fish lateral line

IV. Lectures by Guests

J. Byrne	Quantitative aspects of defensive gill withdrawal in *Aplysia*
C. Levinthal	Development of neural connections in isogenic animals
C. Levinthal	Possible approaches to environmentally induced chemical teratogenesis
E. Kravitz	Octopamine and synaptic modulation in lobster nervous system
G. Fischbach	Studies of nerve and muscle formation and synapse formation in cell culture
J. Dowling	The functional organization of the vertebrate retina
K. Roeder	Input, afference and response; the tactics of bat-evasion by certain moths
K. Roeder	Central processing and integration of acoustic afference in noctuid moths

THE BIOSPHERE

I. Instructors

George M. Woodwell, Brookhaven National Laboratory, and the Ecosystems Center, Marine Biological Laboratory
Daniel Botkin, Yale University and the Ecosystems Center, Marine Biological Laboratory
Charles A. S. Hall, Cornell University and the Ecosystems Center, Marine Biological Laboratory

II. Assistants

David Juers, Brookhaven National Laboratory
Jerry Melillo, Yale University

III. Special Lecturers

K. O. Emery, Woods Hole Oceanographic Institution
Holger Jannasch, Woods Hole Oceanographic Institution
John Teal, Woods Hole Oceanographic Institution
Ivan Valiela, Boston University Marine Program

Robert H. Whittaker, Cornell University
K. Turekian, Woods Hole Oceanographic Institution
Howard Sanders, Woods Hole Oceanographic Institution
Bilal Haq, Woods Hole Oceanographic Institution
William Odum, University of Virginia
W. A. Reiners, Dartmouth College
E. Carpenter, Woods Hole Oceanographic Institution
J. Ballard, Brookhaven National Laboratory
E. Bell, Massachusetts Institute of Technology
R. Hennemuth, National Marine Fisheries Service, Woods Hole
F. Smith, Harvard University
John Adams, Natural Resources Defense Council
Angus MacBeth, Natural Resources Defense Council
F. H. Bormann, Yale University

IV. Lectures

J. D. Ebert	Introduction to the MBL
G. M. Woodwell	The biosphere: a set of interacting ecosystems
G. M. Woodwell	The history of the earth
K. Turekian	Dating of sediments
H. Sanders	Natural communities and evolutionary strategies
K. O. Emery	Sea floor spreading
B. Haq	Paleo-biogeography and climatology of the Atlantic Basin
G. M. Woodwell	The vegetation of the earth
C. A. S. Hall	Flax Pond
C. A. S. Hall	Lakes of the world
J. Teal	World oceans
G. M. Woodwell	The metabolism of the earth
G. M. Woodwell	Energy and carbon in ecosystems
I. Valiela	Experimental analyses of structure and function of a salt marsh
R. H. Whittaker	The evolution of diversity in plant communities
R. H. Whittaker	Gradient analysis
R. H. Whittaker	Indirect ordination
R. H. Whittaker	Evolution of communities
W. Odum	Trophic structure: terrestrial to marine
C. A. S. Hall	Patterns of photosynthesis and migration: New Hope Creek
C. A. S. Hall	Patterns of photosynthesis and migration: salmon
W. A. Reiners	The world carbon budget
D. Botkin	Net primary production of the earth
D. Botkin	Effects of disturbance
G. M. Woodwell	Basic ecology from world-wide pollution
E. Carpenter	Nutrient cycles I
H. Jannasch	Nutrient cycles II
J. Ballard	Experimental eutrophication of terrestrial and aquatic ecosystems with sewage
F. H. Bormann	Biogeochemical cycles I
F. H. Bormann	Biogeochemical cycles II
F. H. Bormann	Succession on land
G. M. Woodwell	The limits of growth: is there an answer in limitless energy?
C. A. S. Hall	Energy flows of man and nature

C. A. S. Hall	The Hudson River and power
G. M. Woodwell	Toxic substances and ecological cycles
E. Bell	World food problem
R. Hennemuth	The future of fish
F. Smith	Urban ecosystems
F. Smith, K. O. Emery	The economics of oil
J. Adams, A. Macbeth	Environment and law
J. Adams, A. Macbeth	New forces of change

THE LABORATORY STAFF

Homer P. Smith, *General Manager*

Teresa M. Braun, Asst. Editor, The Biological Bulletin
Edward G. Casey, Controller
Jane Fessenden, Librarian
A. Robert Gunning, Superintendent, Buildings and Grounds
Jim A. Hancock, Manager, Research Services
Catherine Herrity-Tashiro, Asst. Editor, The Biological Bulletin
Lewis M. Lawday, Asst. Manager, Supply Department
John J. Valois, Manager, Supply Department
Frank A. Wildes, Controller

DIRECTOR'S OFFICE

Marie Abbott, Curator Gray Museum
Lucena J. Barth
Francis P. Bowles, Coordinator of Research Services
Pamela J. Foose
Kathryn Helfrich
Edward F. MacNichol, Jr., Asst. Director for Research Services
Charles A. Ossola, Asst. Director for Finance and Development
Joan M. Upton
George M. Woodwell, Asst. Director for Education and Director of the Ecosystems Center

GENERAL MANAGER'S OFFICE

Florence S. Butz
Agnes L. Geggatt
Elaine C. Perry

CONTROLLER'S OFFICE

Edward J. Bender
Shirley Delisle
Nancy L. Ellis
Joan E. Howard
Lorraine A. Ruddick
Mary R. Tavares

LIBRARY

Judith A. Ashmore
Rebecca J. Bailey
Martha Coldwell
David J. Fitzgerald
Charlotte Goudreau
Joan Grice
Catherine Herrity-Tashiro
Lenora Joseph
Holly Karalekas
Theresa K. McKee
Janet Miller
M. Ann White

MAINTENANCE OF BUILDINGS AND GROUNDS

Eldon P. Allen
Lee E. Bourgoin
James S. Clarke
John V. Day
Manuel P. Dutra
Glenn R. Enos
Charles K. Fuglister
Elizabeth J. Geggatt
Richard E. Geggatt, Jr.
Robert A. Hinz
Ann E. Josephson
Marcia Josephson
Roberta M. Kinnecom
Thomas N. Kleindinst
Elisabeth Kuil
Valea LaBranche
Donald B. Lehy
Ralph H. Lewis
William M. Lochhead
Daniel Loewus
Richard C. Lovering
Alan G. Lunn
John E. Maurer
Stephen Mills
Glenn I. Shear
Lillian A. Stetson
Frederick E. Thrasher
Frederick E. Ward
Ralph D. Whitman
William Whittaker

DEPARTMENT OF RESEARCH SERVICE

Franklin D. Barnes
John Barnes
Julie A. Caissie
Gail Cavanaugh
Christine A. Lehy
Lowell V. Martin
Frank E. Sylvia

SUPPLY DEPARTMENT

Edward G. Enos, Jr.
Joyce B. Enos
David Graham
John H. Ryther, Jr.
A. Dickson Smith
Eugene A. Tassinari
Bruno F. Trapasso
John M. Varao
Frederick W. von Arx

3. Investigators, Lillie, Grass, and Rand Fellows; Students

Independent Investigators, 1975

Abramof, Ita R. Kaiserman, Associate Professor of Anatomy, Case Western Reserve Medical School
Adelman, William J., Chief, Laboratory of Biophysics, National Institutes of Health
Allen, Robert Day, Professor, Chairman, Department of Biological Sciences, Dartmouth College
Amiram, Grinvald, Postdoctoral, Yale University School of Medicine
Anderson, Peter J., Associate Professor, University of Ottawa, Canada
Armstrong, Clay M., Professor of Physiology, University of Rochester
Armstrong, Peter B., Associate Professor of Zoology, University of California, Davis
Arnold, John M., Associate Professor of Cytology, Kewalo Marine Laboratory, University of Hawaii
Bachi, Thomas, University of Zurich, Switzerland
Ball, Eric G., Professor Emeritus of Biological Chemistry, Harvard Medical School
Banerjee, Shib Das, Research Associate, Stanford University Medical School
Barker, Jeffery L., Medical Officer, Public Health Service, National Institutes of Health
Barrett, Dennis, Assistant Professor, University of Denver
Barrett, Martha A., Lecturer, University of Colorado
Battelle, Barbara-Anne, Research Fellow, Department of Neurobiology, Harvard Medical School

Bauer, G. Eric, Associate Professor of Anatomy, University of Minnesota
Beale, Samuel I., Postdoctoral Fellow, The Rockefeller University
Beauge, Luis A., Associate Professor of Biophysics, University of Maryland School of Medicine
Bennett, M. V. L., Professor of Neurosciences, Albert Einstein College of Medicine
Berg, Carl J., Jr., Assistant Professor of Biology, City College, The City University of New York
Bernard, Gary D., Associate Professor of Ophthalmology and Visual Sciences, Associate Professor of Engineering and Applied Science, Yale University School of Medicine
Bernfield, Merton R., Associate Professor of Pediatrics, Stanford University
Bertrand, D., Guest Worker, National Institutes of Health
Bezanilla, Francisco, Professor, Faculty of Sciences, University of Chile, Chile
Bishop, Stephen H., Assistant Professor of Biochemistry, Baylor College of Medicine
Blaustein, Mordecai P., Associate Professor, Washington University School of Medicine
Borgese, Thomas A., Associate Professor of Biology, Herbert Lehman College, The City University of New York
Borisy, Gary G., Associate Professor of Molecular Biology and Zoology, University of Wisconsin
Brinley, F. J., Jr., Associate Professor of Physiology, The Johns Hopkins University
Brodwick, Malcolm S., Assistant Professor, University of Texas, Medical Branch
Brown, Frank A., Jr., Morrison Professor of Biology, Northwestern University
Brown, Joel E., Professor of Anatomy, Vanderbilt University
Bunde, Terry Allan, Postdoctoral Fellow, Baylor College of Medicine
Burdick, Carolyn J., Associate Professor of Biology, Brooklyn College, The City University of New York
Burger, Max M., Chairman of the Biocenter, Biochemistry, University of Basel Biocenter, Switzerland
Burgess, David R., Postdoctoral Fellow and Research Associate, University of Washington, Friday Harbor Laboratories
Cahalan, Michael D., Postdoctoral Fellow, University of Rochester
Cebra, John J., Professor of Biology, The Johns Hopkins University
Chamberlain, John, Assistant Professor, University of Michigan
Chang, Donald C., Assistant Professor, Baylor College of Medicine
Charlton, J. Sherwood, Research Scientist, Marine Biological Laboratory
Claude, Philippa, Principal Research Associate, Harvard Medical School
Clay, John R., Research Associate, University of California, Berkeley
Cloud, Joseph G., Postdoctoral Fellow, The Johns Hopkins University
Cohen, Lawrence B., Associate Professor, Yale University School of Medicine
Cohen, Seymour S., Professor of Microbiology, University of Colorado School of Medicine
Cole, Kenneth S., Research Biophysicist, Laboratory of Biophysics, National Institutes of Health
Condeelis, John S., Postdoctoral Fellow, Dartmouth College
Cooperstein, Sherwin J., Professor of Anatomy, University of Connecticut
Costello, Donald Paul, Kenan Professor of Zoology, University of North Carolina at Chapel Hill
Cremer, G., Priv. Dozent, University of Münster, Germany
Crowe, John H., Assistant Professor of Zoology, University of California, Davis
Cunningham, Patricia, Research Assistant, North Carolina State University
DeTerra, Noel, Assistant Member, The Institute for Cancer Research
DeWeer, Paul, Associate Professor of Physiology and Biophysics, Washington University, School of Medicine
DiPolo, Reinaldo, Associate Investigator, Instituto Venezolano de Investigaciones, Venezuela
Dodge, Frederick A., Adjunct Associate Professor, The Rockefeller University
Dowling, John E., Professor of Biology, Harvard University
Driscoll, Egbert G., Professor of Geology, Wayne State University
DuBois, Arthur B., Professor of Epidemiology and Physiology and Director, John B. Pierce Foundation Laboratory, Yale University Medical School and John B. Pierce Foundation Laboratory
Eaton, Douglas C., Assistant Professor, University of Texas, Medical Branch
Edds, Kenneth T., Postdoctoral Fellow, Marine Biological Laboratory
Edds, Louise Luckenbill, Assistant Professor, Smith College

Ehrenstein, Gerald, Research Physicist, Laboratory of Biophysics, National Institutes of Health
Emmons, Lyman R., Professor of Biology, Washington and Lee University
Epel, David, Professor of Biology, Scripps Institution of Oceanography
Farb, David, Postdoctoral Fellow, Muscular Dystrophy Association, Harvard Medical School
Farmanfarmaian, A., Professor of Physiology, Rutgers University
Fischbach, Gerald, Associate Professor of Pharmacology, Harvard Medical School
Fishman, Harvey M., Associate Professor of Physiology and Biophysics, University of Texas, Medical Branch
Fohlmeister, Jurgen, Lecturer, Postdoctoral Associate, University of Minnesota
Frazier, John M., Assistant Professor of Environmental Medicine, The Johns Hopkins University
French, Robert J., Visiting Fellow, National Institutes of Health
Frishkopf, Lawrence S., Professor of Electrical and Bioengineering, Massachusetts Institute of Technology
Froehner, Stanley C., Research Fellow, Harvard Medical School
Fujiwara, Keigi, Research Fellow in Anatomy, Harvard Medical School
Fuortes, M. G. F., Chief, Laboratory of Neurophysiology, National Institutes of Health
Furshpan, Edwin J., Professor of Neurobiology, Harvard Medical School
Gainer, Harold, Head, Section on Functional Neurochemistry, Behavioral Biology Branch, National Institutes of Health
Gellos, George J., Associate Professor of Biology, Bloomsburg State College
Gelperin, Alan, Associate Professor of Biology, Princeton University
Gibson, Jane, Associate Professor of Biochemistry, Cornell University
Gilbert, Daniel L., Research Physiologist, National Institutes of Health
Giudice, Giovanni, Full Professor, Dean of the Faculty of Sciences, University of Palermo, Italy
Gold, Kenneth, Research Ecologist, New York Zoological Society
Goldsmith, Timothy H., Professor and Chairman, Department of Biology, Yale University
Goldstein, Moise H., Professor of Electrical Engineering, Associate Professor of Biomedical Engineering, The Johns Hopkins University
Greenberg, Michael J., Professor, Florida State University
Grisell, Ronald D., Research Associate, University of Texas, Medical Branch
Grosch, Daniel S., Professor of Genetics, North Carolina State University
Guttman, Rita, Professor of Biology, Brooklyn College, The City University of New York
Hall, Zach W., Associate Professor, Harvard University
Halvorson, Harlyn, Professor of Biology, Director of the Rosenstiel Bascis Medical Sciences Research Center, Brandeis University
Harding, Clifford V., Professor and Director of Research, Kresge Eye Institute, Wayne State University
Hartzell, H. Criss, Research Fellow in Neurobiology, Harvard Medical School
Haschemeyer, Audrey E. V., Professor of Biology and Biochemistry, Hunter College, The City University of New York
Hayes, Raymond L., Associate Professor of Anatomy and Cell Biology, Acting Chairman, University of Pittsburgh
Henley, Catherine, Research Associate, University of North Carolina
Hess, Rainier, Neurophysiologist, Max Planck Institute for Biophysical Chemistry, West Germany
Heuser, John, Assistant Professor of Physiology, University of California, San Francisco
Highstein, Stephen M., Assistant Professor of Neuroscience, Albert Einstein College of Medicine
Hironaka, Tetsuji, Postdoctoral Research Associate, Duke University Medical Center
Hopkins, Penny M., Research Fellow, The American Museum of Natural History
Hoskin, Francis C. G., Professor of Biology and Acting Chairman, Illinois Institute of Technology
Huang, P. C., Associate Professor, The Johns Hopkins University
Huang, R. C., Associate Professor, The Johns Hopkins University
Hubbard, Ruth, Professor of Biology, Harvard University
Humphreys, Tom, Associate Professor, University of Hawaii
Ikegami, Susumu, Bio-Medical Fellow, The Population Council, The Rockefeller University
Ilan, Joseph, Associate Professor, Case Western Reserve University

Ilan, Judith, Assistant Professor, Case Western Reserve University
Inoue, Isao, Visiting Fellow, National Institutes of Health
Jeffery, William R., Assistant Professor, University of Houston
Johnson, James Dean, Staff Assistant, Scripps Institution of Oceanography
Johnson, Ralph G., Professor, University of Chicago
Joyner, Ronald W., Assistant Professor of Physiology, Duke University
Jumblatt, James E., Research Assistant, University of Basel, Switzerland
Kaminer, Benjamin, Professor and Chairman, Boston University School of Medicine
Kammer, Ann E., Associate Professor, Kansas State University
Kanatani, Haruo, Associate Professor, Ocean Research Institute, University of Tokyo, Japan
Kandel, Eric, Professor, Columbia University
Kaneko, Chris R. S., Postdoctoral Fellow, Albert Einstein College of Medicine
Kaplan, Ehud, Postdoctoral Fellow, The Rockefeller University
Kennedy, Mary B., Postdoctoral Fellow, Harvard Medical School
Klein, Paul A., Associate Professor of Pathology, University of Florida
Kochert, Gary, Associate Professor of Botany, University of Georgia
Koide, Samuel S., Associate Director, The Population Council, The Rockefeller University
Kravitz, Edward A., Professor of Neurobiology, Harvard Medical School
Kriebel, Mahlon E., Assistant Professor of Physiology, State University of New York, Upstate Medical Center
Kuffler, Stephen W., John Franklin Enders University Professor, Harvard Medical School
Kusano, Kiyoshi, Professor, Illinois Institute of Technology
Laderman, Aimlee D., Instructor, Ramapo College of New Jersey
Landowne, David, Assistant Professor, University of Miami
Langley, Kenneth H., Associate Professor of Physics, University of Massachusetts
Lanners, Norbert, Postdoctoral Fellow, The Rockefeller University
Lasek, Raymond J., Associate Professor, Case Western Reserve University
Leadbetter, E. R., Professor of Biology, Amherst College
Lee, John J., Professor, City College, The City University of New York
Lester, Roger, Professor of Medicine, University of Pittsburgh School of Medicine
Levinthal, Cyrus, Professor, Columbia University
Levitan, Herbert, Associate Professor, University of Maryland
Levy, Milton, Professor of Biochemistry, New York University School of Medicine
Lipicky, Raymond John, Professor of Pharmacology, Professor of Medicine, and Director of Clinical Pharmacology, University of Cincinnati
Lisman, John, Assistant Professor, Brandeis University
Liuzzi, Anthony, Associate Professor, Lowell Technological Institute
Llinas, R., Professor of Physiology and Biophysics, University of Iowa
Loewénstein, W. R., Professor and Chairman, Department of Physiology and Biophysics, University of Miami School of Medicine
Lorand, L., Professor of Biochemistry and Molecular Biology, Northwestern University
Lyman, Harvard, Associate Professor, State University of New York at Stony Brook
Mann, Diana W., Visiting Assistant Professor of Neurophysiology, Institute of Marine Biomedical Research, University of North Carolina
Mastroianni, Luigi, Jr., William Goodell Professor and Chairman, Department of Obstetrics and Gynecology, University of Pennsylvania
Mathews, Rita W., Research Associate, Hunter College, The City University of New York
Mauzerall, David, Professor, The Rockefeller University
McDonald, Kent L., SEM Technician, University of Colorado
McMahon, Robert F., Assistant Professor of Biology, University of Texas, Arlington
Metuzals, J., Professor in charge of the Electron Microscopy Unit, University of Ottawa, Canada
Metz, Charles B., Professor, University of Mami
Mitchell, Steven W., Instructor of Geology, Mount Holyoke College
Mittenthal, Jay E., Assistant Professor, Purdue University
Moore, John W., Professor of Physiology, Duke University
Moore, Lee E., Associate Professor, Case Western Reserve University
Mote, Michael I., Associate Professor of Biology, Temple University
Mueller, Paul, Medical Research Scientist, Eastern Pennsylvania Psychiatric Institute

Mullins, L. J., Professor of Biophysics and Chairman, University of Maryland School of Medicine
Murphy, Douglas B., Postdoctoral Fellow, University of Wisconsin
Narahashi, Toshio, Professor and Vice-Chairman, Department of Physiology and Pharmacology, Duke University Medical Center
Nelson, Leonard, Professor and Chairman, Department of Physiology, Medical College of Ohio
Neto, Francisco Riccioppo, Postdoctoral Fellow, Duke University Medical Center
Nielsen, Jennifer B. K., Research Associate, Hunter College, The City University of New York
Noe, Bryan D., Assistant Professor of Anatomy, Emory University
Ogelsby, Larry C., Associate Professor of Zoology, Pomona College
Ohki, Shinpei, Associate Professor of Biophysical Sciences, State University of New York at Buffalo
O'Lague, Paul H., Instructor in Neurobiology, Harvard Medical School
Oman, Charles M., Assistant Professor, Massachusetts Institute of Technology
Oxford, Gerry S., Postdoctoral Research Fellow, Duke University Medical Center
Pant, Harish C., Research Fellow, National Institute of Mental Health
Pappas, George D., Professor of Neuroscience and Anatomy, Albert Einstein College of Medicine
Pardy, Rosevelt L., Lecturer, Department of Developmental and Cell Biology, Assistant Dean, University of California, Davis
Parmentier, James L., Postdoctoral Research Fellow, Duke University Medical Center
Pearlman, Alan L., Associate Professor of Physiology and Neurology, Washington University School of Medicine
Person, Philip, Medical Investigator, Veteran's Administration Hospital, Brooklyn
Pfohl, Ronald J., Assistant Professor, Miami University
Pierce, Sidney K., Associate Professor, University of Maryland
Pinto, Lawrence H., Assistant Professor, Purdue University
Pollard, Harvey B., Senior Investigator and Medical Officer, Public Health Service, National Institutes of Health
Pollard, Thomas D., Assistant Professor, Harvard Medical School
Potter, David D., Professor of Neurobiology, Harvard Medical School
Poussart, Denis, Associate Professor, Université Laval, Canada
Powers, Dennis, Assistant Professor, The Johns Hopkins University
Prendergast, Robert A., Associate Professor of Pathology and Ophthalmology, The Johns Hopkins University
Prosen, Edward J., Research Chemist, National Bureau of Standards
Prosser, C. Ladd, Professor of Physiology, University of Illinois, Urbana
Przybylski, Ronald J., Associate Professor, Case Western Reserve University
Rakic, Pasko, Senior Research Associate, Children's Hospital Medical Center
Ram, Jeffrey L., Postdoctoral Fellow, University of California, Santa Cruz
Ramon, Fidel, Assistant Adjunct Professor, Duke University
Ramus, Joseph S., Associate Professor, Yale University
Rankin, Mary Ann, Assistant Professor, University of Texas, Austin
Reese, Thomas S., Head, Section on Functional Neuroanatomy, National Institutes of Health
Reinisch, Carol L., Research Associate, Sidney Farber Cancer Center, Harvard Medical School
Revel, Jean-Paul, Professor, California Institute of Technology
Reynolds, George T., Professor of Physics, Princeton University
Rheuben, Mary B., Postdoctoral Fellow, Yale University
Rice, Robert V., Professor and Head, Department of Biological Sciences, Mellon Institute of Science, Carnegie-Mellon University
Ripps, Harris, Professor of Ophthalmology and Physiology, New York University School of Medicine
Rose, Birgit, Research Assistant Professor, University of Miami School of Medicine
Rosenbaum, Joel L., Associate Professor of Biology, Yale University
Rosenberg, Paul A., Student, Albert Einstein College of Medicine
Roslansky, Priscilla F., Investigator, Carnegie-Mellon University
Ross, William N., Research Fellow, Yale University School of Medicine
Russell, John M., Assistant Professor, University of Texas, Medical Branch
Russell-Hunter, W. D., Professor of Zoology, Syracuse University
Rustad, Ronald C., Associate Professor of Radiology, Anatomy and Biology, Case Western Reserve University

SALZBERG, BRIAN M., Lecturer, Yale University Medical School
SATTELLE, DAVID B., Lecturer, Staff Scientist ARC Unit, Cambridge University, England
SCHOPF, THOMAS J. M., Associate Professor, University of Chicago
SCHUEL, HERBERT, Associate Professor of Biochemistry, State University of New York, Downstate Medical Center
SCHUETZ, ALLEN W., Associate Professor, The Johns Hopkins University
SEARS, JAMES R., Assistant Professor of Biology, Southeastern Massachusetts University
SEE, Y. P., Research Associate, University of Ottawa, Canada
SEGAL, SHELDON J., Vice President, The Population Council, The Rockefeller University
SELMAN, KELLY, Assistant Professor, University of Florida
SENFT, JOSEPH P., Associate Professor, Juniata College
SEYAMA, ISSEI, Postdoctoral Research Associate, Duke University Medical Center
SHARMA, SANSAR C., Assistant Professor, New York Medical College
SHILO, MOSHE, Head of Department of Microbiological Chemistry, Medical School, The Hebrew University, Israel
SHRIVASTAV, BRIJ BHUSHAN, Adjunct Assistant Professor, Duke University Medical Center
SIEBENGA, ELIAS, Research Associate, University of Texas, Galveston
SMITH, FREDERICK E., Professor of Resources and Ecology, Harvard University
SMITH, MICHAEL ANTHONY, Assistant Professor, American University of Beirut, Lebanon
SOBEL, MATTHEW J., Associate Professor, Yale University
SPANGLER, STANLEY G., Postdoctoral Research Fellow, The Johns Hopkins University School of Medicine
SPIEGEL, MELVIN, Professor of Biology, Dartmouth College
SPIRA, M. E., Research Associate, Albert Einstein College of Medicine
SPRAY, DAVID, Research Fellow, Albert Einstein College of Medicine
STARZAK, MICHAEL E., Assistant Professor, State University of New York at Binghamton
STEINACHER, ANTOINETTE, Postdoctoral Fellow, Albert Einstein College of Medicine
STEINHARDT, RICHARD A., Associate Professor of Zoology, University of California, Berkeley
STEPHENS, R. E., Associate Professor and Resident Investigator, Brandeis University, Marine Biological Laboratory
STEPHENSON, WILLIAM K., Professor of Biology and Department Chairman, Earlham College
STETTEN, DEWITT, JR., Deputy Director for Science, National Institutes of Health
STETTEN, MARJORIE R., Biochemist, National Institutes of Health
STOREY, KENNETH B., Assistant Professor of Zoology, Duke University
STRACHER, ALFRED, Professor and Chairman, State University of New York, Downstate Medical Center
STRETTON, ANTONY O. W., Associate Professor, University of Wisconsin
STUART, ANN ELIZABETH, Assistant Professor, Harvard Medical School
STUNKARD, HORACE W., Research Associate, American Museum of Natural History
SZENT-GYORGYI, ALBERT, Director and Principal Investigator, Institute for Muscle Research, Marine Biological Laboratory
SZENT-GYORGYI, ANDREW G., Professor of Biology, Brandeis University
SZENT-GYORGYI, EVA M., Research Associate, Brandeis University
TAKASHIMA, SHIRO, Associate Professor, University of Pennsylvania
TASAKI, ICHIJI, Chief, Laboratory of Neurobiology, National Institutes of Health
TAYLOR, ROBERT E., Research Physiologist, National Institutes of Health
TICKLE, CHERYLL ANNE, Lecturer, Middlesex Hospital Medical School
TIFFERT, TERESA, Research Associate, The Johns Hopkins University School of Medicine
TILNEY, LEWIS G., Associate Professor, University of Pennsylvania
TRELSTAD, ROBERT L., Assistant Professor of Pathology, Harvard Medical School
TRINKAUS, JOHN PHILIP, Professor of Biology, Yale University
TROLL, WALTER, Professor, New York University Medical Center
TROXLER, ROBERT F., Associate Professor of Biochemistry, Boston University School of Medicine
VINCENT, W. S., Professor and Chairman of Biological Sciences, University of Delaware
WITTENBERG, JONATHAN B., Professor of Physiology, Albert Einstein College of Medicine
WARD, SAM, Assistant Professor of Biochemistry, Harvard Medical School
WARASHINA, AKIRA, Visiting Fellow, National Institutes of Health
WARREN, LEONARD, Professor of Therapeutic Research, University of Pennsylvania
WATKINS, DUDLEY T., Associate Professor, University of Connecticut Health Center

Wald, George Higgins, Professor of Biology, Harvard University
Wallace, Robin A., Staff Member, Biology Division, Oak Ridge National Laboratory
Walsby, A. E., University Lecturer, University of North Wales, United Kingdom
Weber, A., Investigator, University of Pennsylvania
Weidner, Earl, Assistant Professor, Louisiana State University
Weissmann, Gerald, Professor of Medicine, New York University Medical Center
Whittaker, J. Richard, Associate Member, Wistar Institute
Wolf, Don P., Assistant Professor, University of Pennsylvania
Wolfe, Ralph S., Professor, University of Illinois
Woodward, William R., Instructor, Harvard Medical School
Wu, Chau H., Adjunct Assistant Professor, Duke University Medical Center
Wyse, Gordon A., Associate Professor of Zoology, University of Massachusetts
Yates, Ida, Research Associate, University of Georgia
Yau, William M., Assistant Professor, Southern Illinois University
Yeh, J. Z., Adjunct Assistant Professor, Duke University Medical Center
Yonemoto, Wesley M., Research Associate, University of Hawaii
Yoshikami, Doju, Instructor in Neurobiology, Harvard Medical School
Zigman, Seymour, Associate Professor of Ophthalmology and Biochemistry, University of Rochester, Medical School

Lillie Fellow, 1975

Okazaki, Kayo, Associate Professor, Tokyo Metropolitan University, Japan

Grass Fellows, 1975

Frazier, D. T., Professor, Senior Fellow, University of Kentucky Medical School
Boron, Walter F., Trainee, Washington University
Coyer, Philip E., Graduate Student, University of Massachusetts
Evans, Peter D., Research Fellow in Neurobiology, Harvard Medical School
Katz, Michael J., Graduate Student, Case Western Reserve University
Kraig, Richard P., Grass Foundation Fellow, University of Iowa
Morgan, Kathleen, Beginning Investigator, University of Cincinnati
Narins, Peter M., Grass Fellow, Cornell University
Requena, Jaime, Associate Investigator, Instituto Venezolano de Investigaciones, Venezuela
Senseman, David Michael, Graduate Student, Princeton University
Sheridan, Robert E., Graduate Student, California Institute of Technology
Siegler, Melody, University of California, Santa Cruz
Swann, John W., Research Physiologist, Armed Forces Radiobiology Research Institute
Teeter, John, Research Fellow, Albert Einstein College of Medicine
Wiese, Konrad, Postdoctoral Fellow, Stanford University

Rand Fellow, 1975

Wehner, Rudiger, Professor, Head of Section of Neurobiology, University of Zurich, Switzerland

Summer Research Scholarships, 1975
(Steps Toward Independence)

Beale, Samuel I.
Berg, Carl J., Jr.
Burgess, David R.
Frazier, John M.
Jeffrey, William R.
Mann, Diana W.
Mittenthal, Jay E.
Oman, Charles M.
Pinto, Larry
Ram, Jeffrey L.
Ramon, Fidel
Reinisch, Carol L.
Rheuben, Mary
Sharma, S. C.
Yau, William M.

Research Assistants, 1975

Anderson, David, Harvard University
Antonellis, Blenda, Case Western Reserve University

Athey, George F., Kansas State University
Baginski, Richard M., University of Maryland
Ballmer, Jurt, University of Basel, Switzerland
Barba, William, Duke University Medical Center
Barkalow, Derek T., Rutgers—The State University
Bennett, Holly Vander Laan, Wheaton College
Berman, Richard, University of Cincinnati
Binder, Robert L., University of Pennsylvania
Black, Paul L., The Johns Hopkins University
Bohr, Vilhem, University of Copenhagen, Denmark
Bosler, Robert B., Harvard Medical School
Boyer, Larry F., University of Chicago
Byers, Hugh Randolph, University of Colorado
Campbell, Susan C., University of Connecticut Medical School
Campisi, Judith, State University of New York at Stony Brook
Carniol, Paul J., John B. Pierce Foundation Laboratory
Caron, Joan M., Yale University
Ciborowski, C. James, Wayne State University
Chow Chong, P., University of Ottawa, Canada
Colbert, Jennifer C., National Bureau of Standards
Collins, Tucker, Amherst College
Cooperstein, Larry, University of Rochester Medical School
Daly, Douglas C., University of Connecticut Medical School
Demcheck, Dennis K., Louisiana State University
Donati, Francois, University of Toronto, Canada
Duckworth, Diana L., University of Chicago
Dunbar, Bonnie S., University of Miami
Duncan, Roger, University of Hawaii
Dutton, Alan R., University of Chicago
Eisner, Yvonne, Cornell University
Ellisman, Mark H., University of Colorado
Emmons, Rebecca P., University of Virginia
Epstein, Kerry, University of Iowa
Erickson, Carol Anne, Yale University
Faison, Brendlyn D., Albert Einstein College of Medicine
Feldman, Lance, University of Cincinnati
Forbes, Douglass Jane, University of Oregon
French, Kathleen A., Mount Vernon College
Green, David J., University of Massachusetts, Amherst
Greenwald, Mark J., Harvard Medical School
Hauger, Steven H., Harvard Medical School
Henderson, Joseph V., State University of New York at Buffalo
Hudson, Alan Paul, The City University of New York
Hurst, Terry W.
Ierardi, Lynn A., Rutgers—The State University
Jampel, Henry, Kresge Eye Institute, Wayne State University
Johnson, Thor S., Brown University
Johnston, Richard L., University of Connecticut
Kaufmann, Karl, University of Chicago
Keeter, Joe S., Albert Einstein College of Medicine
Kohn, Norman Vita, Yale University School of Medicine
Kordik, Ellen R., Illinois Institute of Technology
Kravitz, Jeffrey B., Herbert Lehman College, The City University of New York
LaFratta, James M., Harvard Medical School
Lahey, Karen A., State University of New York at Stony Brook
Lash, Rebecca
Larrinva, Ignacio, State University of New York at Stony Brook
Lauer, Joyce, Harvard Medical School
Lowenhaupt, Manuel T., Massachusetts Institute of Technology
Madin, Katherine Alice Chambers, University of California, Davis

Markey, Carolyn H., University of Massachusetts, Amherst
Martin, Barbara J., University of Connecticut Health Center
Martin, Francis G., Temple University
Mastropaola, Carmine, City College, The City University of New York
Matteson, Donald R., State University of New York, Upstate Medical Center
McBride, Ellen Leigh
McCall, William A., Jr., Wayne State University
Metz, Edward Charles
Moore, Marilyn R., University of Connecticut Health Center
Morales, Eleanor A., New York Aquarium
Morris, James T.
Morris, Robert, Yale University
Murphy, Dennis J., University of Maryland
Narahashi, Keiko, Duke University
Nelson, Robert M., University of Pittsburgh
Noveck, Milton A., Herbert Lehman College, The City University of New York
Osborn, Maria LaGrange, San Francisco State University
Otto, Joann J., University of California, Irvine
Pell, Deborah, University of Texas, Medical Branch
Perry, George, Scripps Institution of Oceanography
Perry, John Gavin, Washington University
Persell, Roger, Hunter College, The City University of New York
Peterson, Scott K., Massachusetts Institute of Technology
Pratt, Melanie M., Brandeis University
Price, Rebecca B., Florida State University
Ragozzino, Mark, University of Lowell
Rayport, Stephen, Harvard University
Reed, Charlene, Florida State University
Reed, Sharon L., The Johns Hopkins University
Rice, Charles M., California Institute of Technology
Rice, Lee R., Carnegie-Mellon University
Robertson, Lola E., American Museum of Natural History
Romesser, James A., University of Illinois
Rossi, Michael W., Case Western Reserve University
Sadun, Alfredo, Albert Einstein College of Medicine
Santiago, Eligio M., Washington University
Schairer, John O., Albert Einstein College of Medicine
Scholl, James E., University of Michigan
Schuetz, Joan, Towson State College
Scruggs, Virginia, University of Miami
Sharp, Gregory H., Duke University
She, Joseph, University of Toronto, Canada
Shishido, Roxanne, Northwestern University
Siegel, Ruth E., Harvard Medical School
Smith, Lee, University of Rochester Medical School
Soodak, Robert, State University of New York at Albany
Starkus, John G., Duke University Medical Center
Stich, Thomas J., The Johns Hopkins University
Swenson, R. P., Jr., Duke University Medical Center
Taylor, Barbara A., Brooklyn College, The City University of New York
Thacker, Sherry V., Emory University
Tuna, Ishik C., The Johns Hopkins University
Turetsky, Oxana, State University of New York at Stony Brook
Verret, Reynold C., Columbia University
Ward, Nathalie F.
Watterson, Nina A., Duke University
Waloga, Geraldine, Harvard University
Walrond, John P., University of Wisconsin

WATSON, PATRICIA, The Johns Hopkins University
WEIGEL, NANCY, The Johns Hopkins University
WESTERFIELD, MONTE, Duke University
WILTSE, WENDY I., University of Massachusetts
WODLINGER, HAROLD M., University of Toronto
WOOD, LOIS ANN, University of Massachusetts
YULO, M. TERESA, University of Rochester, Medical School
ZAKEVICIUS, JANE, New York University School of Medicine
ZIOMEK, CAROL ANN, The Johns Hopkins University

Library Readers, 1975

ADELBERG, EDWARD A., Professor of Human Genetics, Yale University
ALPEN, GARLAND E., Associate Professor of Biology, Washington University
ATLAS, SUSAN J., Postdoctoral Research Fellow, University of Maryland
BELL, EUGENE, Professor of Biology, Massachusetts Institute of Technology
BOWEN, FLORRY P., Research Associate Professor of Neurology, Mt. Sinai School of Medicine, Veteran's Administration Hospital, Bronx
BRANTON, DANIEL, Professor of Biology, Harvard University
CARLSON, FRANCIS D., Professor of Biophysics, The Johns Hopkins University
CHAMBERS, EDWARD L., Professor of Physiology and Biophysics, University of Miami
CHILD, FRANK M., Professor of Biology and Chairman of the Department, Trinity College, Hartford
CHURCHILL, FREDERICK B., Associate Professor of History and Philosophy of Science, Indiana University
CLIFFORD, SISTER ADELE, Professor of Biology, College of Mount St. Joseph on the Ohio
COBB, JEWEL PLUMMER, Dean of the College and Professor of Zoology, Connecticut College
COLEMAN, WILLIAM, Professor of History of Science and Humanistic Studies, The Johns Hopkins University
COLWIN, ARTHUR L., Adjunct Professor, University of Miami
COLWIN, LAURA HUNTER, Adjunct Professor, University of Miami
EDER, HOWARD A., Professor of Medicine, Albert Einstein College of Medicine
FEINMAN, RICHARD D., Assistant Professor, State University of New York, Downstate Medical Center
FIREMAN, PHILIP, Associate Professor of Pediatrics, Director, Allergy—Immunology, University of Pittsburgh School of Medicine and Children's Hospital
GABRIEL, MORDECAI L., Dean, School of Science, Brooklyn College, The City University of New York
GERMAN, JAMES L., III, Senior Investigator and Director, Laboratory of Human Genetics, The New York Blood Center and Cornell University Medical College
GOUDSMIT, ESTHER MARIANNE, Assistant Professor of Biological Sciences, Oakland University
GREEN, JAMES W., Acting Dean of the Graduate School and Professor of Physiology, Rutgers—The State University
HANDLER, PHILIP, President, National Academy of Sciences
HINSCH, GERTRUDE W., Associate Professor, University of South Florida
HUNTER, R. DOUGLAS, Assistant Professor of Biological Sciences, Oakland University
INOUE, SADAYUKI, Assistant Professor, McGill University, Canada
ISENBERG, IRVIN, Professor of Biophysics, Oregon State University
ISSELBACHER, KURT J., Mallinckrodt Professor of Medicine, Chief, Gastrointestinal Unit, Massachusetts General Hospital
KALTENBACH, JANE C., Professor of Biological Sciences, Mount Holyoke College
KARUSH, FRED, Professor of Microbiology, University of Pennsylvania, School of Medicine
KIRSCHENBAUM, DONALD M., Associate Professor, State University of New York Downstate Medical Center
KLEIN, MORTON, Professor of Microbiology, Temple University Medical School
KOSOWER, EDWARD M., Professor of Chemistry, Tel-Aviv University, Israel
LASH, JAMES W., Professor of Anatomy, University of Pennsylvania, School of Medicine

Laster, Leonard, Vice President and Dean, College of Medicine, State University of New York, Downstate Medical Center
Leighton, Joseph, Professor and Chairman, Department of Pathology, Medical College of Pennsylvania
Limoges, Camille, Associate Professor and Institute Director, University of Montreal, Canada
Marsland, Douglas A., Research Professor Emeritus, New York University
McIntosh, J. Richard, Associate Professor, University of Colorado
Mizell, Merle, Professor of Biology, Tulane University
Morrell, Frank, Professor of Neurological Sciences, Rush Medical College
Morrison, Martin, Professor of Biochemistry, St. Jude Children's Research Hospital, The University of Tennessee Center for the Health Sciences
Newbury, Thomas K., Assistant Professor, University of Hawaii
Nicholls, John G., Professor of Physiology, Stanford University School of Medicine
O'Farrell, Helen K., Assistant Professor, Fairleigh Dickinson University
Oschman, James L., Assistant Professor of Biological Sciences, Northwestern University
Palmer, John D., Chairman, Department of Zoology, University of Massachusetts
Porter, Keith Roberts, Professor, University of Colorado
Prusch, Robert D., Assistant Professor of Biology, Brown University
Reiner, John M., Research Professor of Pathology, Professor of Biochemistry, Albany Medical College of Union University
Rosenberg, Evelyn Kevy, Professor of Biology, Jersey City State College
Rosenkranz, Herbert S., Professor of Microbiology, Columbia University
Rowland, Lewis P., Professor and Chairman, Department of Neurology, Columbia University
Rubinow, Sol I., Professor of Biomathematics, Cornell University Medical College; Member, Sloan-Kettering Institute
Rushforth, Norman B., Professor and Chairman, Department of Biology, Case Western Reserve University
Saunders, John W., Professor of Biology, State University of New York at Albany
Schlesinger, R. Walter, Professor and Chairman, Department of Microbiology, Rutgers University Medical School; College of Medicine and Dentistry of New Jersey
Schwartz, James H., Professor of Physiology, Columbia University
Scott, Alwyn C., Professor of Electrical and Computer Engineering, University of Wisconsin
Shedlovsky, Theodore, Professor Emeritus, The Rockefeller University
Shemin, David, Professor of Biochemistry; Chairman, Department of Biochemistry and Molecular Biology, Northwestern University
Sherman, Irwin W., Professor of Zoology, University of California, Riverside
Sly, William S., Associate Professor of Pediatrics and Medicine, Washington University School of Medicine
Sonnenblick, B. P., Professor of Zoology and Physiology, Rutgers—The State University
Speck, William T., Assistant Professor of Pediatrics, Columbia University
Terebey, Nicholas, Assistant Professor, New York University, College of Dentistry
Trager, William, Professor, The Rockefeller University
Tweedell, Kenyon S., Professor, University of Notre Dame
Wall, Betty J., Research Associate, Northwestern University
Wainio, Walter, Professor of Biochemistry, Rutgers—The State University
Waite, Thomas D., Assistant Professor of Civil Engineering, University of Miami
Waksman, Byron, Professor of Immunology, Yale University
Webb, H. Marguerite, Professor of Biological Sciences, Goucher College
Weiss, Leon, Professor, The Johns Hopkins University School of Medicine
Wheeler, George E., Professor of Biology, Brooklyn College, The City University of New York
Wilson, Thomas H., Professor of Physiology, Harvard Medical School
Wittenberg, Beatrice A., Assistant Professor of Physiology, Albert Einstein College of Medicine
Yaphe, W., Professor, McGill University, Canada
Yntema, Chester L., Professor Emeritus of Anatomy, State University of New York, Upstate Medical Center
Yow, Frank, Professor of Biology, Kenyon College
Zacks, Sumner I., Neuropathologist and Professor of Pathology, Pennsylvania Hospital and University of Pennsylvania School of Medicine

Students, 1975

All students listed completed the formal course program. Asterisk indicates completing post-course research program.

Summer Programs 1975

EMBRYOLOGY

Adair , Wyn Steven
Alderton, Janet M.
Anderson, James M.
Atherton, Blair T.
Bates, William R.
Byrd, E. William, Jr.
Cartwright, Joiner
Croissant, Richard
Fraser, Brock R.
Glabe, Charles G.
Griepp, Eva B.
Holton, Beatrice
Kalderon, Nurit
Keller, Thomas C. S.
Magnani, John L.
Pratt, Melanie M.
Radice, Gary P.
Robinson, Kenneth R.
Rodermel, Steven R.
Stoughton, Robert L.
Weller, Nancy K.
Woodrum, Diane T.
Wright, Samuel
Yaross, Marcia S.

EXPERIMENTAL INVERTERBRATE ZOOLOGY

Aldridge, David W.
Allen Richard M.
*Benzer, Martha J.
*Bierman, James F.
*Bleiweiss, Robert
*Cooley, Lynn
*Crawford, Dana R.
*Crowther, Robert
Dawson, Margaret A.
*Farley, Katherine K.
*Galick, Heather A.
Greco, Julie A.
Heacox, Albert E.
*Heathcote, Ralph D.
*Henry, Charles
Horowicz, Carol A.
Hosmer, Hillary A.
Kunen, Eve
Lindan, Christina P.
Marx, Myron
*Oatis, Jonathan W.
O'Donnell, Betty N.
*Ohman, Mark D.
Orzack, Deborah S.
*Parsons, James B.
Ranyard, John R.
*Ready, Neal E.
Reiss, Paul M.
*Ribnik, Linda R.
Shadle, Paula J.
Twigg, Gary G.
Vollmer, Sara
Ward, Nathalie F. R.
White, Roy L.

EXPERIMENTAL MARINE BOTANY

Bergum, Peter W.
Brown, Susan C.
Caldwell, Kendra L.
Cloern, James E.
Denney, Frances R.
Dunlap, Jay C.
Finkle, Joseph M.
Garbary, David
Kilar, John A.
Kinberg, Judy-Lynn
Pearson, Nancy J.
Sternberg, Hal
Wood, Anne M.
Zollner, Jennifer D.

ECOLOGY

Bierbaum, Rosina M.
Bray, Elizabeth A.
Brousseau, Diana J.
Buenning, Inge
Cardillo, Sr. Frances M.
Caumartin, Susan M.
Collier, Ries S.
Countryman, Donald A.
Herman, Ira M.
*Kelly, Charles J., Jr.
Lanyon, Cynthia H.
*Levine, Jeffrey M.
Pederson, Judith
Spaeth, Stephen C.
Stryeski, Kathleen
Wu, Lilian Shiao-Yen
Wetzler, Richard
Zasac, Roman N.

NEUROBIOLOGY

Bodick, Neil C.
Clark, Hal C.
Corwin, Jeffrey T.
Ebner, Timothy J.
Englander, Sol W.
Fernandez, Salvador M.
Hatten, Mary E.
Huttner, Susanne L.
Jabaily, Joseph A.
Livingstone, Margaret S.
Mudge, Anne W.
Price, Donald L.

PHYSIOLOGY

Allen, Pamela L.
*Beswick, David R.
Bleicher, Paul A.
Cook, Wendy D.
D'Amore, Patricia A.
*Detmers, Patricia A.
*Drabkin, Harold J.
*Fechheimer, Marcus
Glad, Richard W.
Goodson, Sylvia S.
Gourlie, Brian B.
Jacobs, John W.
Kiehart, Daniel P.
Krasnow, Richard A. C.
*Lambert, Drew T.
Landercasper, Jeffrey
Lincoln, David W. II
Lowy, Howard W.
Markowitz, Charles B.
Michel, Rene P.
Muhoberac, Barry B.
Nussenzweig, Michel
*Peluso, Richard W.
Singer, Mark S.
Smith, Deborah K.
*Smith, Glenn D.
*Snow, Ernest C., Jr.
*Stanchfield, James E.
Steinbrook, Robert L.
Swenberg, Charles E.
*Wiesmann, Martina L.
Wu, Carl
*Ziegler, H. Kirk

January Programs 1975

BEHAVIOR

Abrams, Judith
Adams, Barbara B.
Babcock, Robert A.
Carr, Barbara A.
Dawis, Dolores M.
Hayes, Marie J.
Hollingshead, Susan K.
Huben, Pauline T.
Johnson, Cynthia A.
Levine, Regina E.
Makay, Debra A.
Moleski, Teresa G.
Platou, Andrea L.
Power, Mary E.
Roanowicz, John D.
Taylor, Elizabeth L.
Volpe, Lane C.
Ward, Nathalie

DEVELOPMENTAL BIOLOGY

Bates, Wiillam R.
Bensky, Norman D.
Caldwell, Constance A.
Crowther, Robert J.
Cruickshank, James A.
Gabrielson, Rick D.
Gentilcore, Peggy M.
Haag, Mary M.
Henry, Charles H.
Kowalski, Mark S.
Kylberg, Heidi K.
Lewis, Marie D.
Loughlin, Jeanne E.
MacLarty, Jan L.
Misley, Paula A.
Moss, David E.
Munson, Katherine E.
Soodak, Robert E.
Suchoff, Daniel S.
Van Dersal, June E.

NEUROBIOLOGY

Bergamini, Michael V. W.
Coleman, Bernard D.
Costello, Walter J.
Coyer, Philip E.
Dearry, C. Allen
Ferguson, Norman B. L.
Gibson, Daniel G. III
Glynn, Paul
Idoniboye-Obu, Birinengi
Iordanidis, Pantazis A.
Jacobson, Richard D.
Johnson, Bruce R.
Kramer, Sanford N.
Krikorian, Debra J.
Lowenhaupt, Manuel T.
Medeiros, John M.
Najarian, Kenneth E.
Nicholas, Bertram A.
Schachter, Debbie C.
Sharnoff, Mark
Sherry, James M.
Stafstrom, Carl E.
Umbach, Joy A.

BIOSPHERE

Boone, Richard D.
Botwick, Charles H.
Brown, Timothy B.
Cooley, Lynn
Fidrocki, Alfred P. I.
Frapwell, Philip D.
Haas, Linda A.
Hamburg, Steven P.
Holz, George G.
Howarth, Robert
Joseph, Cheryl A.
Kaplan, Warren
Klimkowski, Jacqueline L.
Kuhn, Barbara J.
McKenna, James
Potter, Steven C.
Staresinic, Nick
Van Dover, Cindy L.
Waite, Douglas
Warner, Hans J.
Wysolmerski, Sr. Theresa

January Program 1974

(This list of students was omitted from the Annual Report for 1974.)

DEVELOPMENTAL BIOLOGY

Atwood, Kimball C., IV
Baldwin, James D.
Barnard, Ronald M.
Bloom, Kerry S.
Brunt, Melanie
Buss, Leo W.
Cicoria, Anthony D.
Duffner, David W.
Eccles, Sharilyn A.
Frank, James R.
Hennessey, Catherine
Keller, Charles E.
Kelley, Mary-Beth
McLaughlin, Meredith G.
Molnar, Joseph A.
Norberg, Denise R.
Phister, Julia
Plasman, Barbara
Salm, Raymond W., III
Simpson, Ellen
Smith, Wendy A.
Sparks, T. Flint
Stoller, James R.
Thackara, Jeffrey W.
Tracy, Sharon E.
Weiss, Cathy L.
Weiss, Christine L.

4. Fellowships and Scholarships, 1975

Bio Club Scholarship:

Eve Kunen

Gary H. Calkins Scholarship:

Heather A. Galick

Lucretia Crocker Scholarship:

James E. Cloern

5. Training Programs

Research Program in Experimental Marine Botany

I. Consultants

Frank A. Loewus, Chairman, Agricultural Chemistry, Washington State University
Ralph S. Quatrano, Associate Professor of Botany, Oregon State University

II. Senior Investigators

Gary Kochert, Associate Professor of Botony, University of Georgia
Joseph S. Ramus, Associate Professor of Biology, Yale University
Jerome A. Schiff, Professor of Biology, Brandeis University, director of program
Robert F. Troxler, Associate Professor of Biochemistry, Boston University School of Medicine
Anthony E. Walsby, Lecturer in Marine Biology, University of North Wales, United Kingdom

III. Associate Investigators

Samuel I. Beale, Research Associate, The Rockefeller University
Hans Gaffron, Professor Emeritus, University of Florida
Aimlee D. Laderman, Instructor, Ramapo College of New Jersey
David Mauzerall, Professor, The Rockefeller University
Ida Yates, Research Associate, University of Georgia

IV. Trainees

Michael M. Bartolf, Jr.
Martha L. Cullander
Michael Fitzgerald
Wayne D. Frasch
Neil G. Grant
James E. Jurgenson
Jack K. Monroe
Barbara A. Triplett
Raymond E. Tully
Jessie S. Weistrop

V. Lectures

Hans Gaffron	Photosynthesis based on sulfur
Gary Kochert	Developmental mechanisms in *Volvox*
Neil Grant	Respiration of *Chlorella*
Barbara Triplett	Binding studies of wheat germ RNA polymerase II to DNA
Wayne Frasch	*In vivo* biosynthesis of acylated sterol glucosides in tobacco
Raymond Tully	Structure and function of protein bodies during seed germination
John Garbary	Culture of *Ceramium rubrum*
Aimlee Laderman	Synecology of the white cedar swamp: *Euglena* sanguinea bloom carotenoids in peats
Martha Crouch	Mechanisms of self-incompatibility in angiosperms
Richard Ellis	Regulation of chlorophyll synthesis in the green alga *Golenkinia*
Frank Hoskin	Isethionate—an unusual sulfonate anion of the squid giant axon
David Shemin	Porphyrin biosynthesis
David Shemin	δ-aminolevulinic acid dehydratase: structure, function, mechanism
James Jurgenson	Some thoughts on chloroplast genome interactions in tobacco
Carl Price	"Isopycnic sedimentation by surface charge, you say! Be serious, Dr. Pertoft, and listen to the venerable bead."
Jerome A. Schiff	Photocontrol of chloroplast differentiation in *Euglena*
Seymour Cohen	Polyamines in algae
Moshe Shilo	Oxygenic and anoxygenic photosynthesis in *Oscillatoria*
David Mauzerall	Recent advances in photosynthesis
Norman Krinsky	Carotenoid protection against photodamage
Joseph Ramus	Biogenesis of cell surface polysaccharides in red algae
Jerome A. Schiff	Pathways of sulfate reduction in algae
Robert Troxler	Synthesis of phycobiliproteins

Lionel Jaffe	Polarization of *Fucus* eggs
Kent McDonald	Mitosis in diatoms and red algae
David Mauzerall	Protein structure in the purple membrane

Training Program in Microbial Ecology

I. Consultants

Harlyn O. Halvorson, Brandeis University
J. Woodland Hastings, Harvard University
Roger Y. Stanier, Institut Pasteur, Paris
Edward O. Wilson, Harvard University

II. Instructors

Jane Gibson, Cornell University
Holger W. Jannasch, Woods Hole Oceanographic Institution, director of program
Edward R. Leadbetter, Amherst College
Moshe Shilo, Hebrew University, Jerusalem
Ralph S. Wolfe, University of Illinois, Urbana

III. Research Associates

James A. Romesser, University of Illinois, Urbana
Craig D. Taylor, Woods Hole Oceanographic Institution

IV. Trainees

William E. Balch
David P. Brown
David W. Cook
Caroline S. Harwood
Susan B. Leschine
Thomas T. Moench
James D. Pirie
Craig W. Rice
William T. Smorczewski
Judith C. Vogt

V. Lectures

H. W. Jannasch	Introduction to microbial ecology
H. W. Jannasch	Quantitative approaches in microbial ecology
H. W. Jannasch	Theory and practice of the chemostat
H. W. Jannasch	Continuous culture in microbial ecology
R. S. Wolfe	Anaerobic microbial ecology—an overview
R. S. Wolfe	Biochemical basis of anaerobic microbial ecology I
R. S. Wolfe	Biochemical basis of anaerobic microbial ecology II
E. R. Leadbetter	How to make a living aerobically
E. R. Leadbetter	Principles of the microbial enrichment culture
E. R. Leadbetter	The tooth surface as a microbial habitat
E. R. Leadbetter	Nitrate reduction in microorganisms
J. Gibson	Introduction to the photosynthetic bacteria
J. Gibson	Problems in measuring uptake of nutrients in microorganisms
M. Shilo	Introduction to the Bdellovibrio
M. Shilo	Blue green algal viruses
M. Shilo	Bdellovibrio II
M. Shilo	Control and management of algal blooms
M. Shilo	Oxygenic and anoxygenic photosynthesis in Oscillatoria
J. C. Goldman	Continuous culture of photosynthetic organisms
M. M. Allen	The ecology of blue green algae
M. M. Allen	The physiology of blue green algae
A. E. Walsby	Ecology of planktonic blue green algae
R. P. Blakemore	Ecology of marine Spirochaetes
A. E. Walsby	Buoyancy mechanisms in blue green and other algae

C. D. Taylor	The biology of methane formation
R. P. Blakemore	Ecology of marine Spirochaetes
R. S. Wolfe	Magnetotaxis and phototaxis
W. Yaphe	Ecological studies on agar-decomposing bacteria
A. L. Demain	The formation and function of antimicrobial compounds
C. Inderlied	Bacterial enzyme evolution
L. N. Ornston	Evolution of a catabolic pathway
G. E. Jones	Microbial sulfur transformations in Oyster Pond
J. Wilkinson	The methane-oxidizing bacteria
A. E. Walsby	Buoyancy of bacteria
J. S. Poindexter	The procaryotic Brostheca: a structure in search of a niche
M. Levandowsky	Chemoreception in protozoa
J. H. Ryther	Aquaculture

Research Program in Reproductive Biology

I. Instructors

John Chamberlain, University of Michigan
Haruo Kanatani, University of Tokyo, Japan
Charles Metz, University of Miami
Richard Steinhardt, University of California, Berkeley
Joel Rosenbaum, Yale University
Robin Wallace, Oak Ridge National Laboratory, director of program

II. Research Associate

Kelly Selman, University of Florida

III. Course Assistant

Joanne Silberner

IV. Trainees

Theodore Clark
Roger Cochran
Rebecca Ellison
William Gordon
Linda Griffith
Nancy Kravitz
William Marsluff
Lee Opresko
Mary Riederer-Henderson
Patricia Saling
John Valentich
Susan Womann

V. Lectures

R. Goldman	Mechanisms of non-muscle motility
G. Kochert	Sexual differentiation in *Volvox*
D. Phillips	Hybridization of sperm with mammalian cells in culture
D. Wolf	Sperm-egg interaction in the mouse
B. Storey	Metabolism of rabbit spermatozoa

6. Tabular View of Attendance, 1971–1975

	1971	*1972*	*1973*	*1974*	*1975*
Investigators—Total	554	561	523	508	511
Independent	322	328	312	302	301
Library Reader	76	76	86	75	81
Research Assistants	156	157	125	131	129

STUDENTS—TOTAL	130	119	123	146	200
Invertebrate Zoology	29	38	32	30	34
Embryology	28	19	20	21	24
Physiology	33	31	41	40	33
Experimental Botany	22	14	15	11	14
Ecology	18	17	15	14	18
Developmental Biology				30	20
Behavior					17
Biosphere					17
Neurobiology					23
TRAINEES—TOTAL	44	46	50	53	43
TOTAL ATTENDANCE	728	726	696	707	754
Less persons represented in two categories	0	1	0	0	0
	728	725	696	707	754
INSTITUTIONS REPRESENTED—TOTAL	219	210	239	222	237
FOREIGN INSTITUTIONS REPRESENTED	27	25	40	31	26

7. INSTITUTIONS REPRESENTED, 1975

Alabama, University of
Albert Einstein College of Medicine
American Museum of Natural History
Amherst College
Armed Forces Radiobiology Research Institute
Baylor College
Bloomsburg State College
Boston University
Boston University School of Medicine
Brandeis University
Brooklyn College, The City University of New York
Brown University
California Institute of Technology
California, University of, Berkeley
California, University of, Davis
California, University of, Irvine
California, University of, Los Angeles
California, University of, Riverside
California, University of, San Diego
California, University of, San Francisco
California, University of, Santa Cruz
Carleton College
Carnegie-Mellon University
Case Western Reserve University
Case Western Reserve University, Medical School
Children's Hospital Medical Center, Boston
Chicago, University of
Cincinnati, University of
Cincinnati, University of, College of Medicine
City College, The City University of New York
Clark University
College of Mount St. Joseph on the Ohio
Colorado, University of
Colorado, University of, Medical Center
Colorado College
Columbia University
Connecticut, University of
Connecticut, University of, Medical School
Connecticut College
Cornell University
Cornell University Medical College
Dartmouth College
Delaware, University of
Denver, University of
Detroit Board of Education
Duke University
Duke University Medical Center
Earlham College
East Tennessee State University
Emory University
Fairleigh Dickinson University
Florida, University of
Florida State University
Franklin Pierce College
Georgia, University of
Goucher College
Gulf Coast Research Laboratory
Harvard Medical School
Harvard University
Hawaii, University of
Herbert Lehman College, The City University of New York
Houston, University of
Hunter College, The City University of New York
IBM Research, T. J. Watson Research Center
Illinois, University of
Illinois Institute of Technology

Indiana University
Institute for Muscle Research, Woods Hole
Iowa, University of
Jersey City State College
John B. Pierce Foundation Laboratory
Johns Hopkins University, The
Johns Hopkins University, The, School of Hygiene
Johns Hopkins University, The, School of Medicine
Juniata College
Kansas State University
Kansas University
Kentucky, University of
Kenyon College
Kewalo Marine Laboratory
Kirkland College
Kresge Eye Institute, Detroit
Ladycliff College
Lawrence University
Louisiana State University
Lowell, University of
Lowell Technological Institute
Maryland, University of
Maryland, University of, School of Medicine
Massachusetts, University of, Amherst
Massachusetts, University of, Boston
Massachusetts General Hospital
Massachusetts Institute of Technology
Medical College of Pennsylvania
Medical College of Virginia
Mellon Institute of the Carnegie-Mellon University
Miami, University of
Miami, University of, School of Marine and Atmospheric Science
Miami, University of, School of Medicine
Miami University
Michigan, University of
Minnesota, University of
Moravian College
Mount Holyoke College
Mt. Sinai School of Medicine, The City University of New York
National Academy of Science
National Bureau of Standards
National Heart and Lung Institute
National Institute of Arthritis, Metabolism and Digestive Diseases
National Institute of Mental Health
National Institute of Neurological Diseases and Stroke
National Institutes of Health
National Marine Fisheries Service
National Marine Fisheries Service, Milford Laboratory
New College
New Hampshire, University of
New York Blood Center, The
New York Medical College
New York University
New York University College of Dentistry
New York University Medical Center
New York University School of Medicine
New York Zoological Society
North Carolina, University of, at Chapel Hill
North Carolina State University, Raleigh
Northeastern University
Northwestern University
Notre Dame, University of
Oak Ridge National Laboratory
Oakland University
Oberlin College
Ohio State University
Oregon, University of
Oregon State University
Pennsylvania, University of
Pennsylvania, Hospital of the University of
Pennsylvania, University of, School of Medicine
Pittsburgh, University of
Pittsburgh, University of, School of Medicine and Children's Hospital
Pomona College
Population Council, The
Princeton University
Purdue University
Ramapo College of New Jersey
Reed College
Rensselaer Polytechnic Institute
Rice University
Rochester, University of
Rochester, University of, Medical School
Rockefeller University, The
Rush Medical College
Rutgers—The State University
Rutgers University Medical School
St. Anselm's College
St. Francis College
St. Jude Children's Research Hospital
San Francisco State University
Sangamon State University
Scripps Institution of Oceanography
Seton Hall College
Smith College
South Florida, University of
Southeastern Massachusetts University
Southern Illinois University
Stanford University
State University of New York, Downstate Medical Center
State University of New York, Upstate Medical Center
State University of New York at Albany
State University of New York at Binghamton
State University of New York at Buffalo
State University of New York at Oneonta
State University of New York at Purchase

State University of New York at Stony Brook
Syracuse University
Temple University
Temple University Medical School
Texas, University of, Arlington
Texas, University of, Austin
Texas, University of, Medical Branch
Toledo, University of
Towson State College
Trinity College, Hartford
Tulane University
Union University, Albany Medical College of
Vanderbilt University
Vermont, University of
Veteran's Administration Hospital, Bronx
Veteran's Administration Hospital, Brooklyn
Virginia, University of
Washington and Jefferson College
Washington and Lee University
Washington, University of
Washington, University of, Friday Harbor Laboratories
Washington State University
Washington University, St. Louis
Washington University, School of Medicine
Wayne State University
Wellesley College
Wesleyan University
West Florida, University of
Wisconsin, University of
Wistar Institute
Woods Hole Oceanographic Institution
Wyoming, University of
Yale University
Yale University, School of Medicine

Foreign Institutions Represented, 1975

Acadia University, Canada
Alberta, University of, Canada
American University, Beirut, Lebanon
Basel, The University of, Switzerland
Cambridge University, United Kingdom
Chile, University of, Chile
Copenhagen, University of, Denmark
Guelph, University of, Canada
Hebrew University, The, Israel
Instituto Venezolano de Investigaciones, Venezuela
Max Planck Institute for Biophysical Chemistry, West Germany
McGill University, Canada
Middlesex Hospital Medical School, United Kingdom
Montréal, Université de, Canada
Münster, University, Germany
North Wales, University of, United Kingdom
Ocean Research Institute, Japan
Ottawa, University of, Canada
Palermo, University of, Italy
Tel-Aviv University, Israel
Tokyo, University of, Japan
Tokyo Metropolitan University, Japan
Toronto, University of Canada
Université Laval, Canada
Weizmann Institute of Science, Israel
Zurich, University of, Switzerland

8. Friday Evening Lectures, 1975

June 27

F. J. R. Taylor.................Red tides
University of British Columbia

July 4

George M. Woodwell..........Report on the biosphere 1975: How is life?
Marine Biological Laboratory

July 10

Harry Grundfest..............The natural history of neurons
Columbia University,
Alexander Forbes Lecturer at the
Marine Biological Laboratory

July 11

Harry Grundfest..............Excitability of membranes
Columbia University

July 18

Richard L. Sidman Harvard Medical School	Genetic and local environmental cell interactions in cerebellar development

July 25

Lawrence Bogorad Harvard University	Intergenomic cooperation: a principle of organelle biology?

August 1

Cyrus Levinthal Columbia University	Watching nerves find their way—cell shapes and growth patterns in genetically identical animals

August 8

Rüdiger Wehner University of Zurich, Rand Fellow at the Marine Biological Laboratory	E-vector detection and celestial orientation in insects

August 15

Elizabeth Hay Harvard Medical School	Collagen—cell surface interaction in corneal morphogenesis

August 22

Alfred Nisonoff Brandeis University	Idiotypy as a genetic marker for variable regions of immunoglobulin polypeptide chains

9. Members of the Corporation, 1975

Including Action of 1975 Annual Meeting

Life Members

Adolph, Dr. Edward F., University of Rochester School of Medicine and Dentistry, Rochester, New York 14627

Beam, Dr. Harold W., Department of Zoology, State University of Iowa, Iowa City, Iowa 52240

Barth, Dr. Lester G., Marine Biological Laboratory, Woods Hole, Massachusetts 02543

Behre, Dr. Ellinor H., Black Mountain, North Carolina 28711

Bertholf, Dr. Lloyd M., 1228 Gettysburg Drive, Bloomington, Illinois 61701

Bodansky, Dr. Oscar, 16 Hawks Nest Road, Stony Brook, New York 11790

Bridgman, Dr. A. Josephine, 715 Kirk Rd., Decatur, Georgia 30030

Brown, Dr. Dugald E. S., Cape Haze, Box 426, Placida, Florida 33946

Burdick, Dr. C. Lalor, The Lalor Foundation, 4400 Lancaster Pike, Wilmington, Delaware 19805

Clarke, Dr. George L., 44 Juniper Road, Belmont, Massachusetts 02178

Cole, Dr. Elbert C., 2 Chipman Park, Middlebury, Vermont 05753

Crouse, Dr. Helen V., Institute for Molecular Biophysics, Florida State University, Tallahassee, Florida 32306

DILLER, DR. IRENE C., 2417 Fairhill Avenue, Glenside, Pennsylvania 19038
DILLER, DR. WILLIAM F., 2417 Fairhill Avenue, Glenside, Pennsylvania 19038
FERGUSON, DR. JAMES K. W., 56 Clarkson St., Thornhill, Ontario, Canada
FISCHER, DR. ERNST, 3110 Manor Drive, Richmond, Virginia 23230
FRIES, Dr. ERIK F. B., 41 High Street, Woods Hole, Massachusetts 02543
FURTH, DR. JACOB, 99 Fort Washington Ave., New York, New York 10032
GAFFRON, DR. HANS, P. O. Box 308, Sanibel, Florida 33959
GALTSOFF, DR. PAUL S., National Marine Fisheries Service, Woods Hole, Massachusetts 02543
GRAY, DR. IRVING E., Department of Zoology, Duke University, Durham, North Carolina 27701
GRUNDFEST, DR. HARRY, Department of Neurology, Columbia University, College of Physicians and Surgeons, New York, New York 10032
HAMBURGER, DR. VIKTOR, Department of Zoology, Washington University, St. Louis, Missouri 63110
HIBBARD, DR. HOPE, 366 Reamer Place, Oberlin, Ohio 44074
HISAW, DR. F. L., 5925 S. W. Plymouth Drive, Corvallis, Oregon 97330
HOLLAENDER, DR. ALEXANDER, Associated University, Inc., 1717 Massachusetts Ave., N. W. , Washington, D. C. 20036
IRVING, DR. LAURENCE, University of Alaska, College, Alaska 99701
KAAN, DR. HELEN, 62 Locust St., Apt. 244, Falmouth, Massachusetts 02540
KAHLER, ROBERT, Box 423, Woods Hole, Massachusetts 02543
KILLE, DR. FRANK R., 340 Albany Shaker Road, Londonville, New York 12211
LYNN, DR. W. GARDNER, Department of Biology, Catholic University of America, Washington, D. C. 20017
MACDOUGALL, DR. MARY STUART, Mt. Vernon Apartments, 423 Clairmont Avenue, Decatur, Georgia 30030
MAGRUDER, DR. SAMUEL R., Rte. 4, Box 177, Kevil, Kentucky 42053
MALONE, DR. E. F., 6610 North 11th Street, Philadelphia, Pennsylvania 19126
MANWELL, DR. REGINALD D., Department of Biology, Syracuse University, Syracuse, New York 13210
MARSLAND, DR. DOUGLAS, 3523 Loquat Ave., Miami, Florida 33103
MILLER, DR. JAMES A., Department of Anatomy, Tulane University, New Orleans, Louisiana 70112
MOUL, DR. E. T., 42 F. R. Lillie Rd., Woods Hole, Massachusetts 02543
PAGE, Dr. I. H., Cleveland Clinic, Euclid at E. 93rd Street, Cleveland, Ohio 44106
PAYNE, DR. FERNANDUS, Wesley Manor, 1555 N. Main St., Frankfort, Indiana 46041
PLOUGH, DR. H. H., 31 Middle Street, Amherst, Massachusetts 01002
POLLISTER, DR. A. W., Box 23, Dixfield, Maine 04224
POND, SAMUEL E., 53 Alexander Street, Manchester, Connecticut 06044
PORTER, DR. H. C., University of Pennsylvania, Philadelphia, Pennsylvania 19104
RICHARDS, DR. A. GLENN, Department of Entomology, University of Minnesota, St. Paul, Minnesota 55101
RUGH, Dr. ROBERTS, Grosvenor Park, Apt. 1018, 10500 Rockville Pike, Rockville, Maryland 20852
SCHMITT, DR. FRANCIS O., 165 Allen Dale St., Jamaica Plain, Massachusetts 02130
SCHRADER, DR. SALLY, 2717 Dogwood Rd., Durham, North Carolina 27705

Schramm, Dr. J. R., Department of Plant Sciences, Indiana University, Bloomington, Indiana 47401
Severinghaus, Dr. Aura E., 375 West 250th Street, New York, New York 10071
Sichel, Dr. Elsa K., 4 Whitman Rd., Woods Hole, Massachusetts 02543
Smith, Dr. Dietrich C., 216 Oak Forest Ave., Catonville, Maryland 21228
Speidel, Dr. Carl C., 1873 Field Road, Charlottesville, Virginia 22903
Straus, Dr. W. L., Jr., Department of Anatomy, The Johns Hopkins University Medical School, Baltimore, Maryland 21205
Stunkard, Dr. Horace W., American Museum of Natural History, Central Park West at 79th Street, New York, New York 10024
Taylor, Dr. W. Randolph, Department of Botany, University of Michigan, Ann Arbor, Michigan 48104
TeWinkel, Dr. Lois E., 4 Sanderson Ave., Northampton, Massachusetts 01060
Turner, Dr. C. L., Northwestern University, Evanston, Illinois 60201
Waite, Dr. F. G., 144 Locust St., Dover, New Hampshire 03820
Warren, Dr. Herbert S., ℅ Leland C. Warren, 721 Conshohocken State Road, Penn Valley, Pennsylvania 19072
Weiss, Dr. Paul, The Rockefeller University, 66th St. and York Ave., New York, New York 10016
Wichterman, Dr. Ralph, 31 Buzzards Bay Ave., Woods Hole, Massachusetts 02543
Young, Dr. D. B., Main Street, North Hanover, Massachusetts 02357

Regular Members

Abbott, Dr. Bernard C., Department of Biological Sciences, University of Southern California, University Park, Los Angeles, California 90007
Abbott, Dr. Marie B., Resident Systematist, Marine Biological Laboratory, Woods Hole, Massachusetts 02543
Ache, Dr. Barry W., Department of Biological Sciences, Florida Atlantic University, Boca Raton, Florida 33432
Acheson, Dr. George H., 25 Quissett Ave., Woods Hole, Massachusetts 02543
Adelberg, Dr. Edward A., Department of Microbiology, Yale University Medical School, New Haven, Connecticut 06510
Afzelius, Dr. Bjorn, Wenner-Gren Institute, University of Stockholm, Stockholm, Sweden
Allen, Dr. Garland E., Biology Department, Washington University, St. Louis, Missouri 63110
Allen, Dr. Nina S., Department of Biology, Dartmouth College, Hanover, New Hampshire 03755
Allen, Dr. Robert D., Chairman, Department of Biology, Dartmouth College, Hanover, New Hampshire 03755
Alscher, Dr. Ruth, Department of Biology, Manhattanville College, Purchase, New York 10577
Amatniek, Ernest, 154 Bay Road, Huntington, New York 11743
Amberson, Dr. William R., Katy Hatch Road, Falmouth, Massachusetts 02540
Anderson, Dr. Everett, Department of Anatomy and Laboratories of Human Reproductive Biology, Harvard Medical School, Boston, Massachusetts 02115

Anderson, Dr. J. M., Division of Biological Sciences, Emerson Hall, Cornell University, Ithaca, New York 14853

Armstrong, Dr. Clay M., Department of Physiology, University of Rochester, Rochester, New York 14603

Armstrong, Dr. Phillip B., Department of Anatomy, State University of New York, Upstate Medical Center, Syracuse, New York 13210

Arnold, Dr. John Miller, Pacific Biomedical Research Center, 2538 The Mall, University of Hawaii, Honolulu, Hawaii 96822

Arnold, Dr. Dr. William A., Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830

Ashworth, Dr. John M., Department of Geology, University of Essex, Wivenhoe Park, Colchester, C0435Q, England, U. K.

Atwood, Dr. Kimball C., 100 Haven Ave., Apt. 21-E, New York New York 10032

Austin, Dr. Mary L., 506½ North Indiana Avenue, Bloomington, Indiana 47401

Bacon, Robert, Church Street, Woods Hole, Massachusetts 02543

Bakalar, David, 200 Allendale, Jamaica Plains, Massachusetts 02130

Ball, Dr. Eric G., P. O. Box 406, Falmouth, Massachusetts 02541

Bang, Dr. F. B., Department of Pathobiology, The Johns Hopkins University School of Hygiene, Baltimore, Maryland 21205

Bard, Dr. Phillip, Department of Physiology, The Johns Hopkins University Medical School, Baltimore, Maryland 21205

Barker, Dr. Jeffery L., Behavioral Biology Branch, Bldg. 36, Room B-308, NICHD, National Institutes of Health, Bethesda, Maryland 20014

Barlow, Dr. Robert B., Jr., Laboratory of Sensory Research, Syracuse University, 821 University Avenue, Syracuse, New York 13210

Bartell, Dr. Clelmer K., Department of Biological Sciences, Louisiana State University of New Orleans, New Orleans, Louisiana 70113

Barth, Dr. Lucena, Marine Biological Laboratory, Woods Hole, Massachusetts 02543

Bartlett, Dr. James H., Department of Physics, University of Alabama, P. O. Box 1921, University, Alabama 35486

Bauer, Dr. G. Eric, Department of Anatomy, University of Minnesota, Minneapolis, Minnesota 55414

Beauge, Dr. Luis Alberto, Department of Biophysics, University of Maryland School of Medicine, 660 W. Redwood St., Baltimore, Maryland 21201

Beck, Dr. L. V. Department of Pharmacology, Indiana University, School of Experimental Medicine, Bloomington, Indiana 47401

Belamarich, Dr. Frank A., Department of Biology, Boston University, Boston, Massachusetts 02215

Bell, Dr. Allen, RFD#1, Cambridge, Maine 04923

Bell, Dr. Eugene, Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Bennett, Dr. Michael V. L., Department of Neuroscience, Albert Einstein College of Medicine, Eastchester Rd. and Morris Park Ave., New York, New York 10461

Bennett, Dr. Miriam F., Department of Biology, Colby College, Waterville, Maine 04901

Bergstrom, Dr. Beverly H., 115 W. Squantum St., Sagamore Towers #903, North Quincy, Massachusetts 02171

Berman, Dr. Mones, National Institutes of Health, Theoretical Biology nci, Bldg. 10, 4B56, Bethesda, Maryland 20014

Bernard, Gary D., Department of Opthalmology and Visual Science, Yale University, 333 Cedar St., New Haven, Connecticut 06510

Berne, Dr. Robert W., University of Virginia School of Medicine, Charlottesville, Virginia 22903

Bernheimer, Dr. Alan W., New York University College of Medicine, New York, New York 10016

Biggers, Dr. John Dennis, Department of Physiology, Harvard Medical School, 25 Shattuck St., Boston, Massachusetts 02115

Bishop, Dr. David W., Department of Physiology, Medical College of Ohio at Toledo, P. O. Box 6190, Toledo, Ohio 43614

Blanchard, Dr. K. C., The Johns Hopkins University Medical School, Baltimore, Maryland 21205

Blaustein, Mordecai P., Department of Physiology and Biophysics, Washington University School of Medicine, St. Louis, Missouri 63110

Bloch, Dr. Robert, Adalbertstr. 70–8, Munich, Germany (13)

Blum, Dr. Harold F., 612 E. Durham St., Philadelphia, Pennsylvania 19119

Bodian, Dr. David, Department of Otolaryngology, The Johns Hopkins University, 1721 Madison St., Baltimore, Maryland 21205

Boettiger, Dr. Edward G., Department of Zoology, University of Connecticut, Storrs, Connecticut 06268

Bold, Dr. Harold C., Department of Botany, University of Texas, Austin, Texas 78712

Boolootian, Dr. Richard A., President, Science Software System, 11899 West Pico Blvd., Los Angeles, California 90064

Borei, Dr. Hans G., Department of Zoology, University of Pennsylvania, Philadelphia, Pennsylvania 19104

Borgese, Dr. Thomas A., Department of Biology, Lehman College, City University of New York, Bronx, New York 10468

Borisy, Dr. Gary G., Laboratory of Molecular Biology, University of Wisconsin, Madison, Wisconsin 53715

Borsellino, Dr. Antonio, Instituto di Fiscia, Viale Benedetto XV, 5 Genova, Italy

Bosch, Dr. Herman F., Marine Biological Laboratory, Woods Hole, Massachusetts 02543

Bowen, Dr. Vaughn T., Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543

Bowles, Dr. Francis P., Boston University Marine Program, Marine Biological Laboratory, Woods Hole, Massachusetts 02543

Brandt, Dr. Philip Williams, Department of Anatomy, Columbia University, College of Physicians and Surgeons, New York, New York 10032

Brinley, Dr. F. J., Jr., Department of Physiology, The Johns Hopkins University Medical School, Baltimore, Maryland 21205

Brooks, Dr. Matilda M., Department of Physiology, University of California, Berkeley, California 94720

Brown, Dr. Frank A., Jr., Department of Biological Sciences, Northwestern University, Evanston, Illinois 60201
Brown, Dr. Joel E., The Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138
Buck, Dr. John B., Laboratory of Physical Biology, National Institutes of Health, Bethesda, Maryland 20014
Burbanck, Dr. Madeline Palmer, Box 15134, Atlanta, Georgia 30333
Burbanck, Dr. William D., Box 15134, Atlanta, Georgia 30333
Burdick, Dr. Carolyn J., Department of Biology, Brooklyn College, Brooklyn, New York 11210
Burger, Dr. Max M., Department of Biochemistry, University of Basel, CH. 4056-Klingelbergstrasse 70, Basel, Switzerland
Burky, Dr. Albert J., Department of Biology, University of Dayton, Dayton, Ohio 45469
Burnett, Dr. Allison Lee, Department of Biology, Northwestern University, Evanston, Illinois 60201
Candelas, Dr. Graciela C., Department of Biology, University of Puerto Rico, Rio Piedras, Puerto Rico 00931
Carlson, Dr. Francis D., Department of Biophysics, The Johns Hopkins University, Baltimore, Maryland 21218
Carpenter, Dr. Russell L., 60-H Lake Street, Winchester, Massachusetts 01890
Carriker, Dr. Melbourne R., College of Marine Studies, University of Delaware, Field Station, Lewes, Delaware 19958
Case, Dr. James F., Department of Biological Sciences, University of California, Santa Barbara, California 93106
Cassidy, Rev. Joseph P., O.P., Department of Biological Science, OT Hogan Bldg., Northwestern University, Evanston, Illinois 60201
Cebra, Dr. John J., Department of Biology, The Johns Hopkins University, Baltimore, Maryland 21218
Chaet, Dr. Alfred B., University of West Florida, Pensacola, Florida 32506
Chambers, Dr. Edward L., Department of Physiology and Biophysics, University of Miami School of Medicine, P. O. Box 52087, Biscayne Annex, Miami, Florida 33152
Chappell, Dr. Richard L., Department of Biological Sciences, Hunter College of the City University of New York, New York, New York 10021
Chase, Dr. Aurin M., Department of Biology, Princeton University, Princeton, New Jersey 08540
Chauncey, Dr. Howard H., 30 Falmouth Rd., Wellesley Hills, Massachusetts 02181
Cheney, Dr. Ralph H., 11 Park Street, Woods Hole, Massachusetts 02543
Child, Dr. Frank M., Department of Biology, Trinity College, Hartford, Connecticut 06106
Citkowitz, Dr. Elena, 410 Livingston St., New Haven, Connecticut 06511
Clark, Dr. A. M., Department of Biological Sciences, University of Delaware, Newark, Delaware 19711
Clark, Dr. Eloise E., National Science Foundation, 1800 G Street, Washington, D. C. 20550

Clark, Hays, Executive Vice-President, Avon Products, Inc., 9 West 57th Streeet, New York, New York 10019

Clark, Dr. Wallis H., National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Biological Laboratory, 4700 Avenue U., Galveston, Texas 77550

Clayton, Dr. Roderick K., Section of Genetics, Development and Physiology, Cornell University, Ithaca, New York 14850

Clement, Dr. A. C., Department of Biology, Emory University, Atlanta, Georgia 30322

Clowes, Dr. George H. A., Jr., Harvard Medical School, Boston, Massachusetts 02115

Cobb, Dr. Jewel P., Dean of the College, Connecticut College, New London, Connecticut 06320

Cohen, Dr. Adolph I., Department of Ophthalmology, Washington University, School of Medicine, 4550 Scott Ave., St. Louis, Missouri 63110

Cohen, Dr. Lawrence B., Department of Physiology, Yale University, New Haven, Connecticut 06510

Cohen, Dr. Seymour S., Department of Microbiology, University of Colorado Medical School, Denver, Colorado 80220

Cole, Dr. Kenneth S., Laboratory of Biophysics, NINDS, National Institutes of Health, Bethesda, Maryland 20014

Collier, Dr. Jack R., Department of Biology, Brooklyn College, Brooklyn, New York 11210

Colwin, Dr. Arthur L., Division of Functional Biology, University of Miami, School of Marine and Atmospheric Sciences, 10 Rickenbacker Causeway, Miami, Florida 33149

Colwin, Dr. Laura H., Division of Functional Biology, University of Miami, School of Marine and Atmospheric Sciences, 10 Rickenbacker Causeway, Miami, Florida 33149

Cooperstein, Dr. Sherwin J., School of Medicine, University of Connecticut, Farmington, Connecticut 06032

Copeland, Dr. D. Eugene, Department of Biology, Tulane University, New Orleans, Louisiana 70118

Corliss, Dr. John O., Department of Zoology, University of Maryland, College Park, Maryland 20742

Cornell, Dr. Neal W., 1914 Marthas Rd., Alexandria, Virginia 22307

Cornman, Dr. Ivor, 10A Orchard Street, Woods Hole, Massachusetts 02543

Costello, Dr. Donald P., Department of Zoology, University of North Carolina, Chapel Hill, North Carolin 27514

Costello, Dr. Helen Miller, Department of Zoology, University of North Carolina, Chapel Hill, North Carolina 27514

Couch, Dr. Ernest F., Department of Biology, Texas Christian University, Forth Worth, Texas 76110

Cousineau, Dr. Gilles H., Department of Biology, Montreal University, P. O. Box 6128, Montreal, P. Q., Canada

Crane, John O., Box 145, Woods Hole, Massachusetts 02543

Cremer-Bartels, Dr. Gertrud, Universitats Augenklinik, 44 Munster, Germany

Crippa, Dr. Marco, Department de Biologie animale, Embrologie Moleculaire, 154 route de Malagnou, Geneve, Switzerland

Crowell, Dr. Sears, Department of Zoology, Indiana University, Bloomington, Indiana 47401

Daignault, Alexander T., W. R. Grace Co., 1114 Avenue of the Americas, New York, New York 10036

Dan, Dr. Jean Clark, Department of Biology, Ochanomizu University, Otsuka, Bunkyo-Ku, Tokyo, Japan

Dan, Dr. Katsuma, Misaki Biological Station, Misaki, Japan

Danielli, Dr. James F., Life Sciences Department, Worcester Polytechnic Institute, Worcester, Massachusetts 01609

Davidson, Dr. Eric H., Division of Biology, California Institute of Technology, Pasadena, California 91109

Davis, Dr. Bernard D., Harvard Medical School. 25 Shattuck Street, Boston, Massachusetts 02115

Daw, Dr. Nigel W., Department of Physiology, Washington University Medical School, 4566 Scott Avenue, St. Louis, Missouri 63110

DeHaan, Dr. Robert L., Department of Anatomy, Emory University, Atlanta, Georgia 30322

DeLanney, Dr. Louis E., Department of Biology, Ithaca College, Ithaca, New York 14850

DePhillips, Dr. Henry A., Jr., Department of Chemistry, Trinity College, Hartford, Connecticut 06106

Dettbarn, Dr. Wolf-Dietrich, Department of Pharmacology, Vanderbilt University, School of Medicine, Nashville, Tennessee 37217

deVillafranca, Dr. George W., Department of Zoology, Smith College, Northampton, Massachusetts 01060

DeWeer, Dr. Paul J., Department of Physiology, Washington University Medical School, St. Louis, Missouri 63110

Diehl, Dr. Fred Alison, Department of Biology, University of Virginia, Charlottesville, Virginia 22904

Dische, Dr. Zacharias, College of Physicians and Surgeons, Columbia University, 630 W. 165th Street, New York, New York 10032

Dixon, Dr. Keith E., School of Biological Sciences, Flinders University, Bedford Park, South Australia

Doolittle, Dr. R. F., Department of Chemistry, University of California, San Diego, La Jolla, California 92037

Dowdall, Dr. Michael J., Max Planck-Institut fur Biophysikalische Chemie, D-3400 Gottingen, West Germany

Dowling, Dr. John E., Biological Laboratories, Harvard University, 16 Divinity Avenue, Cambridge, Massachusetts 02138

Dresden, Dr. Marc H., Department of Biochemistry, Baylor College of Medicine, Houston, Texas 77025

Dudley, Dr. Patricia L. Department of Biological Sciences, Barnard College, Columbia University, New York, New York 10027

Dunham, Dr. Philip B., Department of Biology, Syracuse University, Syracuse, New York 13210

Ebert, Dr. James David, Department of Embryology, Carnegie Institution of Washington, Baltimore, Maryland 21210

Eckert, Dr. Roger O., Department of Zoology, University of California, Los Angeles, California 90024

Edds, Dr. Kenneth T., Box 348, Woods Hole, Massachusetts 02543

Eder, Dr. Howard A., Albert Einstein College of Medicine, Bronx, New York 10461

Edwards, Dr. Charles, Department of Biological Sciences, State University of New York at Albany, Albany, New York 12203

Együd, Dr. Laszlo G., Biochemical Pharmacology, Division of Biological and Medical Sciences, Brown University, Providence, Rhode Island 02912

Ehrenstein, Dr. Gerald, National Institutes of Health, Bethesda, Maryland 20014

Eichel, Dr. Herbert J., Department of Biochemistry, Hahnemann Medical College, Philadelphia Pennsylvania 19104

Eisen, Dr. Arthur Z., Division of Dermatology, Washington University, School of Medicine, St. Louis, Missouri 63110

Eisen, Dr. Herman, Center for Cancer Research, Department of Biology, Massachusetts Institute of Technology, Rm 56–526, Cambridge, Massachusetts 02139

Elder, Dr. Hugh Young, Institute of Physiology, University of Glasgow, Glasgow, Scotland, U. K.

Elliott, Dr. Gerald F., The O. U. Research Unit, 11/12 Bevington Rd., Oxford, England, U. K.

Epel, Dr. David, Scripps Institute of Oceanography, University of California, San Diego, La Jolla, California 92037

Epstein, Dr. Herman T., Department of Biology, Brandeis University, Waltham, Massachusetts 02154

Erulkar, Dr. Solomon D., Department of Pharmacology, University of Pennsylvania Medical School, Philadelphia, Pennsylvania 19104

Essner, Dr. Edward S., Kresge Eye Institute, Wayne State University, School of Medicine, 540 E. Canfield Ave., Detroit, Michigan 48201

Ettiene, Dr. Earl M., Department of Anatomy, Harvard Medical School, Boston, Massachusetts 02115

Failla, Dr. Patricia M., Office of the Director, Argonne National Laboratory, Argonne, Illinois 60439

Farmanfarmaian, Dr. Allahverdi, Department of Physiology and Biochemistry, Rutgers University, New Brunswick, New Jersey 08903

Faust, Dr. Robert Gilbert, Department of Physiology, University of North Carolina Medical School, Chapel Hill, North Carolina 27514

Fawcett, Dr. D. W., Department of Anatomy, Harvard Medical School, Boston, Massachusetts 02115

Fein, Dr. Alan, Marine Biological Laboratory, Woods Hole, Massachusetts 02543

Ferguson, Dr. F. P., National Institute of General Medical Sciences, National Institutes of Health, Bethesda, Maryland 20014

Fertziger, Dr. Allen P., Department of Physiology, University of Maryland Medical School, Baltimore, Maryland 21201

Fessenden, Jane, Librarian, Marine Biological Laboratory, Woods Hole, Massachusetts 02543

Fine, Dr. Jacob, 576 Contlanza Street, Stanford, California 94305

Fingerman, Dr. Milton, Department of Biology, Tulane University, New Orleans, Louisiana 70118

Fisher, Dr. J. M., Department of Biochemistry, University of Toronto, Toronto 5, Ontario, Canada

Fishman, Dr. Louis, 143 North Grove Street, Valley Stream, New York 11580

Fishman, Dr. Harvey M., Department of Physiology, University of Texas Medical Branch, Galveston, Texas 77550

Flanagan, Dennis, Editor, Scientific American, 415 Madison St., New York, New York 10017

Fox, Dr. Maurice S., Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

Fraenkel, Dr. Gottfried S., Department of Entomology, University of Illinois, Urbana, Illinois 61801

Franzini, Dr. Clara, Department of Anatomy, University of Pennsylvania, School of Medicine, Philadelphia, Pennsylvania 19174

Frazier, Dr. Donald T., Department of Physiology and Biophysics, University of Kentucky, Lexington, Kentucky 40507

Freeman, Dr. Alan R., Professor and Chairman, Department of Physiology, Temple University School of Medicine, 3420 N. Broad St. Philadelphia, Pennsylvania 19140

Freeman, Dr. Gary L., Department of Zoology, University of Texas, Austin, Texas 78710

Freygang, Dr. Walter H., Jr., 6247 29th Street, N. W., Washington, D. C. 20015

Fulton, Dr. Chandler M., Department of Biology, Brandeis University Waltham, Massachusetts 02154

Fuortes, Dr. Michael G. F., National Institute for Neurological Diseases and Stroke, National Institutes of Health, Bethesda, Maryland 20014

Furshpan, Dr. Edwin J., Department of Neurophysiology, Harvard Medical School, Boston, Massachusetts 02115

Fuseler, Dr. John W., Department of Human Biological Chemistry and Genetics, University of Texas Medical Branch, Galveston, Texas 77550

Fye, Dr. Paul M., Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543

Gabriel, Dr. Mordecai L., Department of Biology, Brooklyn College, Brooklyn, New York 11210

Gainer, Dr. Harold, Head, Section of Functional Neurochemistry, National Institutes of Health, Bldg. 36, Rm. B-308, Bethesda, Maryland 20014

Gall, Dr. Joseph G., Department of Biology, Yale University, New Haven, Connecticut 06520

Gelfant, Dr. Seymour, Department of Dermatology, Medical College of Georgia, Augusta, Georgia 30904

Gelperin, Dr. Alan, Department of Biology, Princeton University, Princeton, New Jersey 08540

German, Dr. James L., III, The New York Blood Center, 310 East 67th Street, New York, New York 10021
Gibbs, Dr. Martin, Institute for Photobiology of Cells and Organelles, Brandeis University, Waltham, Massachusetts 02154
Gibson, Dr. A. Jane, Wing Hall, Cornell University, Ithaca, New York 14850
Gifford, Dr. Prosser, Woodrow Wilson Center, Smithsonian Building, Washington, D. C. 20560
Gilbert, Dr. Daniel L., Laboratory of Biophysics, NINCDS, National Institutes of Health, Building 36, Room 2A-29, Bethesda, Maryland 20014
Gilman, Dr. Lauren C., Department of Biology, University of Miami, Coral Gables, Florida 33124
Ginsberg, Dr. Harold S., College of Physicians and Surgeons, Columbia University, 630 W. 168th St., New York, New York 10032
Giudice, Dr. Giovanni, University of Palermo, Via Archirafi 22, Palermo, Italy
Golden, William T., 40 Wall Street, New York, New York 10005
Goldman, David E., Department of Physics and Biophysics, Medical College of Pennsylvania, 3300 Henry Avenue, Philadelphia, Pennsylvania 19129
Goldsmith, Dr. Mary H. M., Department of Biology, Kline Biology Tower, Yale University, New Haven, Connecticut 06520
Goldsmith, Dr. Timothy H., Department of Biology, Yale University, New Haven, Connecticut 06520
Goldstein, Dr. Moise H., Jr., 506 Traylor Bldg., The Johns Hopkins University, School of Medicine, 720 Rutland Ave., Baltimore, Maryland 21205
Gooch, Dr. James L., Department of Biology, Juniata College, Huntingdon, Pennsylvania 16652
Goodchild, Dr. Chauncey G., Department of Biology, Emory University, Atlanta, Georgia 30322
Gorman, Dr. Anthony L. F., 333 Worcester Street, Wellesley, Massachusetts 02181
Gottschall, Dr. Gertrude Y., 315 East 68th Street, Apartment 9M, New York, New York 10021
Goudsmit, Dr. Esther M., Department of Biology, Oakland University, Rochester, Michigan 48063
Graham, Dr. Herbert, 36 Wilson Road, Woods Hole, Massachusetts 02543
Grant, Dr. David C., Department of Biology, Davidson College, Box 2316, Davidson, North Carolina 28036
Grant, Dr. Phillip, Department of Biology, University of Oregon, Eugene, Oregon 97403
Grass, Albert, The Grass Foundation, 77 Reservoir Road, Quincy, Massachusetts 02170
Grass, Ellen R., The Grass Foundation, 77 Reservoir Road, Quincy, Massachusetts 02170
Green, Dr. James W., Department of Physiology, Rutgers University, New Brunswick, New Jersey 08903
Green, Dr. Jonathan P., Laboratory of Comparative Physiology, Department of Zoology, University of Malaya, Kuala Lumpur, Malaysia
Green, Dr. Maurice, Department of Microbiology, St. Louis University Medical School, St. Louis, Missouri 63103

Greenberg, Dr. Michael J., Department of Biological Sciences, Florida State University, Tallahassee, Florida 32306

Gregg, Dr. James H., Department of Zoology, University of Florida, Gainesville, Florida 32601

Greif, Dr. Roger L., Department of Physiology, Cornell University Medical College, New York, New York 10021

Griffin, Dr. Donald R., The Rockefeller University, 66 Street and York Avenue, New York, New York 10021

Grosch, Dr. Daniel S., Department of Genetics, Gardner Hall, North Carolina State University, Raleigh, North Carolina 27607

Grossman, Dr. Albert, New York University Medical School, New York, New York 10016

Gunning, Mr. A. Robert, 377 Hatchville Road, Hatchville, Massachusetts 02536

Guttman, Dr. Rita, Department of Biology, Brooklyn College, Brooklyn, New York 11210

Gwilliam, Dr. G. F., Department of Biology, Reed College, Portland, Oregon 97202

Hall, Dr. Zack W., Department of Biophysics, University of Houston, Houston, Texas 77002

Halvorson, Dr. Harlyn O., Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, Massachusetts 02154

Hamilton, Dr. Howard L., Department of Biology, University of Virginia, Charlottesville, Virginia 22901

Harding, Dr. Clifford V., Jr., Professor and Director of Research, Kresge Eye Institute, Wayne State University, School of Medicine, 540 E. Canfield, Detroit, Michigan 48201

Harrington, Dr. Glenn W., Department of Microbiology, University of Missouri, School of Dentistry, 650 E. 25th Street, Kansas City, Missouri 64108

Hartline, Dr. H. Keffer, The Rockefeller University, 66th Street and York Avenue, New York, New York 10021

Hartman, Dr. H. Bernard, Department of Zoology, University of Iowa, Iowa City, Iowa 52240

Haschemeyer, Dr. Audrey E. V., Department of Biological Sciences, Hunter College, 695 Park Avenue, New York, New York 10021

Hastings, Dr. J. Woodland, The Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138

Hauschka, Dr. T. S., R. F. D. 1, Biscay Pond, Damarescotta, Maine 04543

Haxo, Dr. Francis T., Department of Marine Biology, Scripps Institution of Oceanography, University of California, La Jolla, California 92038

Hayashi, Dr. Teru, 3100 S. Michigan, Chicago, Illinois 60616

Hayes, Dr. Raymond L., Jr., Department of Anatomy and Cell Biology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15219

Hegyeli, Dr. Andrew F., 10824 Middleboro Drive, Damascus, Maryland 20750

Hendley, Dr. Charles D., 615 South Avenue, Highland Park, New Jersey 08904

Henley, Dr. Catherine, 5225 Pooks Hill Road, Apt. 1120 North, Bethesda, Maryland 20014

Herndon, Dr. Walter R., 506 Andy Holt Tower, University of Tennessee, Knoxville, Tennessee 37916

Hervey, John P., Box 8-5 Penzance Road, Woods Hole, Massachusetts 02543
Hessler, Dr. Anita Y., 5795 Waverly Avenue, La Jolla, California 92037
Hiatt, Dr. Howard H., Office of the Dean, Harvard School of Public Health, 55 Shattuck St., Boston, Massachusetts 02115
Highstein, Dr. Stephen M., Division of Cellular Neurobiology, Albert Einstein College of Medicine, Morris Park Avenue, Bronx, New York 14061
Hill, Dr. Robert Benjamin, Department of Zoology, University of Rhode Island, Kingston, Rhode Island 02881
Hillman, Dr. Peter, Department of Biology, Hebrew University, Jerusalem, Israel
Hinegardner, Dr. Ralph T., Division of Natural Sciences, University of California, Santa Cruz, California 95060
Hinsch, Dr. Gertrude W., Department of Biology, University of South Florida, Tampa, Florida 33620
Hodge, Dr. Charles, IV, P. O. Box 4095, Philadelphia, Pennsylvania 19118
Hoffman, Dr. Joseph, Department of Physiology, Yale University School of Medicine, New Haven, Connecticut 06515
Hollyfield, Dr. Joe C., Department of Ophthalmology, Columbia University, 630 W. 168th Street, New York, New York 10032
Holtzman, Dr. Eric, Department of Biological Science, Columbia University, New York, New York 10032
Holz, Dr. George G., Jr., Department of Microbiology, State University of New York, Upstate Medical Center, Syracuse, New York 13210
Hoskin, Dr. Francis C. G., Biology Department, Illinois Institute of Technology, Chicago, Illinois 60616
Houston, Howard, Preston Avenue, Meriden, Connecticut 06450
Hubbard, Dr. Ruth, The Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138
Humes, Dr. Arthur G., Boston University Marine Program, Marine Biological Laboratory, Woods Hole, Massachusetts 02543
Hummon, Dr. William D., Department of Zoology, Ohio University, Athens, Ohio 45701
Humphreys, Dr. Tom D., P. B. R. C.-University of Hawaii, 41 Ahui Street, Honolulu, Hawaii 96813
Hunter, Dr. Bruce, Department of Zoology, Connecticut College, New London, Connecticut 06320
Hunter, Dr. R. Douglas, Department of Biological Sciences, Oakland University, Rochester, Michigan 48063
Hunziker, H. E., Main St., Falmouth, Massachusetts 02540
Hurwitz, Dr. Charles, Basic Science Research Laboratory, VA Hospital, Albany, New York 12208
Hurwitz, Dr. Jerard, Department of Molecular Biology, Albert Einstein College of Medicine, Eastchester Road and Morris Park Avenue, Bronx, New York 10461
Huxley, Dr. Hugh E., Medical Research Council, Laboratory of Molecular Biology, Cambridge, England, U. K.
Hyde, Dr. Beal B., Department of Botany, University of Vermont, Burlington, Vermont 05401

Hyde, L. Robinson, Princeton University, Princeton, New Jersey 08540

Ilan, Dr. Joseph, Department of Anatomy, Case Western Reserve University, School of Medicine, Cleveland, Ohio 44106

Inoue, Dr. Sadayuki, Department of Pathology, Pathology Institute, McGill University, 3775 University Street, Montreal 112, Quebec, Canada

Inoué, Dr. Shinya, 217 Leidy Lab Building, Department of Biology, University of Pennsylvania, Philadelphia, Pennsylvania 19104

Isenberg, Dr. Irving, Department of Biochemistry and Biophysics, Oregon State University, Corvallis, Oregon 97331

Isselbacker, Dr. Kurt J., Massachusetts General Hospital, Boston, Massachusetts 02714

Izzard, Dr. Colin S., Department of Biological Sciences, State University of New York at Albany, Albany, New York 12207

Jacobson, Dr. Antone G., Department of Biology, University of Texas, Austin, Texas 78712

Jaffee, Dr. Lionel, Department of Biology, Purdue University, Lafayette, Indiana 47907

Jannasch, Dr. Holger W., Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543

Jeffery, Dr. William R., Department of Biophysics, University of Houston, Houston, Texas 77002

Jenner, Dr. Charles E., Department of Zoology, University of North Carolina, Chapel Hill, North Carolina 27514

Jennings, Dr. Joseph B., Department of Zoology, University of Leeds, Leeds LS2 9JT, England, U. K.

Johnson, Dr. Frank H., Department of Biology, Princeton University, Princeton, New Jersey 08540

Johnson, Dr. Ralph G., Department of Geophysical Sciences, University of Chicago, Chicago, Illinois 60637

Jones, Dr. E. Ruffin, Jr., Department of Biological Sciences, University of Florida, Gainesville, Florida 32601

Jones, Dr. Meredith L., Division of Worms, Museum of Natural History, Smithsonian Institution, Washington, D. C. 20650

Jones, Dr. Raymond F., Department of Biology, State University of New York at Stony Brook, Long Island, New York 11753

Josephson, Dr. R. K., School of Biological Sciences, University of California, Irvine, California 92664

Kabat, Dr. E. A., Department of Neurobiology, Columbia University, College of Physicians and Surgeons, New York, New York 10032

Kafatos, Dr. Fotis C., The Biological Laboratories, Harvard University, 16 Divinity Avenue, Cambridge, Massachusetts 02138

Kaji, Dr. Akira, Department of Microbiology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104

Kaley, Dr. Gabor, Department of Physiology, Basic Sciences Building, New York Medical College, Valhalla, New York 10595

Kaminer, Dr. Benjamin, Department of Physiology, Boston University School of Medicine, 80 E. Concord St., Boston, Massachusetts 02118

Kammer, Dr. Ann E., Division of Biology, Kansas State University, Manhattan, Kansas 66502

Kane, Dr. Robert E., Pacific Biomedical Research Center, 41 Ahui Street, University of Hawaii, Honolulu, Hawaii 96813

Kaneshiro, Dr. Edna S., Department of Biological Sciences, University of Cincinnati, Cincinnati, Ohio 45221

Karakashian, Dr. Stephen J., 165 West 91st Street, Apt. 16-F, New York, New York 10024

Karush, Dr. Fred, Department of Microbiology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104

Katz, Dr. George M., Department of Neurology, Columbia University, College of Physicians and Surgeons, 630 West 168th Street, New York, New York 10032

Kean, Dr. Edward L., Departments of Biochemistry and Ophthalmology, Case Western Reserve University, Cleveland, Ohio 44101

Kelly, Dr. Robert E., Department of Anatomy, University of Illinois, College of Medicine, P. O. Box 6998, Chicago, Illinois 60680

Kemp, Dr. Norman E., Department of Zoology, University of Michigan, Ann Arbor, Michigan, 48104

Kendall, Mr. John P., One Boston Place, Boston, Massachusetts 02108

Kennedy, Dr. Eugene P., Department of Biological Chemistry, Harvard University Medical School, Boston, Massachusetts 02115

Keosian, Dr. John, P. O. Box 193, Woods Hole, Massachusetts 02543

Ketchum, Dr. Bostwick H., Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543

Keynan, Dr. Alexander, Vice President, Hebrew University, Jerusalem, Israel

King, Dr. Thomas J., Program Director, National Bladder and Prostatic Cancer Programs, Division of Cancer Grants, National Cancer Institute, Westwood Bldg., Rm. 853, Bethesda, Maryland 20014

Kingsbury, Dr. John M., Department of Botany, Cornell University, Ithaca, New York 14850

Kirschenbaum, Dr. Donald, Department of Biochemistry, College of Medicine, State University of New York, 450 Clarkson Avenue, Brooklyn, New York 11203

Klein, Dr. Morton, Department of Microbiology, Temple University, Philadelphia, Pennsylvania 19122

Kleinholz, Dr. Lewis H., Department of Biology, Reed College, Portland, Oregon 97202

Kleyn, Dr. John G., Department of Biology, University of Puget Sound, Tacoma, Washington 98416

Klotz, Dr. I. M., Department of Chemistry, Northwestern University, Evanston, Illinois 60201

Kohler, Dr. Kürt, Biologisches Institut der Universitat Stuttgart, D-7 Stuttgart 60 Ulmer Str. 227, West Germany

Koningsberg, Dr. Irwin R., Department of Biology, Gilmer Hall, University of Virginia, Charlottesville, Virginia 22903

Korr, Dr. I. M., College of Osteopathic Medicine, Michigan State University, East Lansing, Michigan 48824

Krahl, Dr. M. E., Department of Physiology, Stanford University, Stanford, California 94305

Krane, Dr. Stephen M., Massachusetts General Hospital, Boston, Massachusetts 02114

Krassner, Dr. Stuart Mitchell, Department of Developmental and Cell Biology, University of California, Irvine, California 92650

Krauss, Dr. Robert, Dean, School of Science, Oregon State University, Corvallis, Oregon 97330

Kriebel, Dr. Mahlon E., Department of Physiology, State University of New York, Upstate Medical Center, Syracuse, New York 13210

Krieg, Dr. Wendell J. S., 1236 Hinman, Evanston, Illinois 60602

Krupa, Dr. Paul L., Department of Biology, The City College of New York, 139th St. and Convent Avenue, New York, New York 10031

Kuffler, Dr. Stephen W., Department of Neurophysiology, Harvard Medical School, Boston, Massachusetts 02115

Kusano, Dr. Kiyoshi, Biology Department, Illinois Institute of Technology, 3300 South Federal Street, Chicago, Illinois 60616

LaMarche, Dr. Paul H., 593 Eddy St., Providence, Rhode Island 02903

Lamy, Dr. Francois, Department of Biochemistry, University of Sherbrooke, School of Medicine, Sherbrooke, Quebec, Canada

Lancefield, Dr. Rebecca C., The Rockefeller University, 1230 York Ave., New York, New York 10021

Landowne, Dr. David, Department of Physiology, University of Miami, Miami, Florida 33124

Lang, Dr. Frederick, Boston University Marine Program, Marine Biological Laboratory, Woods Hole, Massachusetts 02543

Lansing, Dr. Albert I., Department of Anatomy, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15213

Lash, Dr. James W., Department of Anatomy, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19174

Laster, Dr. Leonard, State University of New York, Downstate Medical Center, 450 Clarkson Ave., Brooklyn, New York 11203

Laufer, Dr. Hans, Biological Sciences Group U-H2, University of Connecticut, Storrs, Connecticut 06268

Lauffer, Dr. Max A., Department of Biophysics, University of Pittsburgh, Pittsburgh, Pennsylvania 15260

Lavin, Dr. George I., 6200 Norvo Road, Baltimore, Maryland 21207

Lawrence, E. Swift, President, Falmouth National Bank, Falmouth, Massachusetts 02540

Leadbetter, Dr. Edward R., Department of Biology, Amherst College, Amherst, Massachusetts 01002

Leak, Dr. Lee Virn, Department of Anatomy, Howard University, College of Medicine, Washington, D. C. 20001

Lecar, Dr. Harold, Laboratory of Biophysics, National Institute of Neurological Diseases and Stroke, National Institutes of Health, Bethesda, Maryland 20014

Lederberg, Dr. Joshua, Department of Genetics, Stanford Medical School, Palo Alto, California 94304

Lee, Dr. John J., Department of Biology, City College of the City University of New York, Convent Avenue and 138th Street, New York, New York 10031
LeFevre, Dr. Paul G., Department of Physiology, State University of New York at Stony Brook, Stony Brook, New York 11790
Leighton, Dr. Joseph, Department of Pathology, Medical College of Pennsylvania, 3300 Henry Ave., Philadelphia, Pennsylvania 19129
Lenher, Dr. Samuel, 1900 Woodlawn Avenue, Wilmington, Delaware 19806
Lerman, Dr. Sidney, Department of Opthalomology, Emory University, Atlanta, Georgia 30322
Lerner, Dr. Aaron B., Yale Medical School, New Haven, Connecticut 06510
Levin, Dr. Jack, Department of Medicine, The Johns Hopkins Hospital, Baltimore, Maryland 21205
Levine, Dr. Rachmiel, 2024 Canyon Road, Arcadia, California 91006
Levinthal, Dr. Cyrus, Department of Biological Sciences, Columbia University, 908 Schermerhorn Hill, New York, New York 10027
Levy, Dr. Milton, 39–95 48th Street, Long Island City, New York 11104
Lewin, Dr. Ralph A., Scripps Institution of Oceanography, La Jolla, California 92037
Lewis, Dr. Herman W., Genetic Biology Program, National Science Foundation, Washington, D. C. 20025
Ling, Dr. Gilbert, 307 Berkeley Road, Merion, Pennsylvania 19066
Linskens, Dr. H. P., Department of Botany, University of Driehuizerweg 200, Nijmegen, The Netherlands
Lipicky, Dr. Raymond J., Department of Pharmacology, College of Medicine, University of Cincinnati, 231 Bethesda Avenue, Cincinnati, Ohio 45267
Little, Dr. E. P., 216 Highland Street, West Newton, Massachusetts 02158
Liuzzi, Dr. Anthony, Department of Radiological Sciences, University of Lowell, Lowell, Massachusetts 01854
Llinas, Dr. Rodolfo R., Department of Physiology and Biophysics, University of Iowa, Iowa City, Iowa 52240
Lochhead, Dr. John H., 49 Woodlawn Rd., London S. W. 6, England, U. K.
Loewenstein, Dr. Werner R., Physiology and Biophysics, School of Medicine, University of Miami, P. O. Box 875, Miami, Florida 33152
Loewus, Dr. Frank A., Department of Agricultural Chemistry, Washington State University, Pullman, Washington 99163
Loftfield, Dr. Robert B., Department of Biochemistry, University of New Mexico Medical School, 900 Stanford N. E., Albuquerque, New Mexico 87106
London, Dr. Irving M., 16-512, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139
Lorand, Dr. Laszlo, Department of Chemistry, Northwestern University, Evanston, Illinois 60201
Love, Dr. Warner E., 4338 North Charles Street, Baltimore, Maryland 21218
Luria, Dr. Salvador E., Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139
Lynch, Dr. Clara J., 4800 Fillmore Avenue, Alexandria, Virginia 22311
MacNichol, Edward F., Jr., Marine Biological Laboratory, Woods Hole, Massachusetts 02543

Mahler, Dr. Henry R., Department of Biochemistry, Indiana University Bloomington, Indiana 47401

Malkiel, Dr. Saul, Children's Cancer Research Foundation, Inc., 35 Binney Street, Boston, Massachusetts 02115

Manalis, Dr. Richard S., Department of Physiology, University of Cincinnati, College of Medicine, Eden and Bethesda Aves., Cincinnati, Ohio 45219

Mangum, Dr. Charlotte P., Department of Biology, College of William and Mary, Williamsburg, Virginia 23185

Marks, Dr. Paul A., Columbia University, College of Physicians and Surgeons, 630 West 168th Street, New York, New York 10032

Marsh, Dr. Julian B., Department of Biochemistry and Physiology, Medical College of Pennsylvania, 3300 Henry Ave., Philadelphia, Pennsylvania 19129

Mautner, Dr. Henry G., Tufts University School of Medicine, 136 Harrison Avenue, Department of Biochemistry and Pharmacology, Boston, Massachusetts 02111

Mauzerall, Dr. David, The Rockefeller University, 66th Street and York Avenue, New York, New York 10021

Maxwell, Dr. Arthur, Provost, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543

Mazia, Dr. Daniel, Department of Zoology, University of California, Berkeley, California 94720

McCann, Dr. Frances, Department of Physiology, Dartmouth Medical School, Hanover, New Hampshire 03755

McCloskey, Dr. Lawrence R., Department of Biology, Walla Walla College, College Place, Washington 99324

McDaniel, Dr. James Scott, Department of Biology, East Carolina College, Greenville, North Carolina 27834

McLaughlin, Jane A., Institute for Muscle Research, Marine Biological Laboratory, Woods Hole, Massachusetts 02543

McMahon, Dr. Robert F., Department of Biology, University of Texas, Arlington, Texas 76019

McReynolds, Dr. John S., Laboratory of Neurophysiology, NINDB, National Institutes of Health, Bethesda, Maryland 20014

Meinkoth, Dr. Norman A., Department of Biology, Swathmore College, Swathmore, Pennsylvania 19081

Mellon, Dr. DeForest, Jr., Department of Biology, University of Virginia, Charlottesville, Virginia 22903

Mendelson, Dr. Martin, Health Sciences Centers, State University of New York, Stony Brook, New York 11790

Metz, Dr. C. B., Institute of Molecular Evolution, University of Miami, 521 Anastasia St., Coral Gables, Florida 33134

Middlebrook, Dr. Robert, Downsway, School Lane, Kirk Ella, Hull, England, U. K. HW10 7NR

Milkman, Dr. Roger D., Department of Zoology, University of Iowa, Iowa City, Iowa 52242

Mills, Dr. Eric Leonard, Institute of Oceanography, Dalhousie University, Halifax, Nova Scotia, Canada

Mills, Robert, 56 Worcester Ct., Falmouth, Massachusetts 02540

Milne, Dr. Lorus J., Department of Zoology, University of New Hampshire, Durham, New Hampshire 03824

Mizell, Dr. Merle, Department of Biology, Tulane University, New Orleans, Louisiana 70118

Monroy, Dr. Alberto, CNR Laboratory of Molecular Embryology, 80072 Arco Felice, Napoli, Italy

Montroll, Dr. Elliott W., Institute for Fundamental Studies, Department of Physics, University of Rochester, Rochester, New York 14627

Moore, Dr. John A., Department of Biology, University of California, Riverside, California 92502

Moore, Dr. John W., Department of Physiology, Duke University Medical Center, Durham, North Carolina 27706

Moran, Dr. Joseph F., Jr., 23 Foxwood Drive, RR#1, Eastham, Massachusetts 02642

Morin, Dr. James G., Department of Zoology, University of California, Los Angeles, California 90024

Morlock, Dr. Noel L., Department of Surgery, Detroit General Hospital, 1326 St. Antoine Street, Detroit, Michigan 48226

Morrill, Dr. John B., Jr., Division of Natural Sciences, New College, Sarasota, Florida 33578

Morse, Dr. Richard Stetson, 193 Winding River Road, Wellesley, Massachusetts 02181

Morse, Robert W., Associate Director, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543

Moscona, Dr. A. A., Department of Zoology, University of Chicago, Chicago, Illinois 60627

Mote, Dr. Michael I., Department of Biology, Temple University, Philadelphia, Pennsylvania 19122

Mountain, Dr. Isabel M., 17 Brookfield Pl., Pleasantville, New York 10570

Mullins, Dr. Lorin J., Department of Biophysics, University of Maryland School of Medicine, Baltimore, Maryland 21201

Musacchia, Dr. Xavier J., Department of Physiology and Space Sciences, University of Missouri Medical School, Columbia, Missouri 65201

Nabrit, Dr. S. M., 686 Beckwith Street S. W., Atlanta, Georgia 30314

Nace, Dr. Paul Foley, 5 Bowditch Rd., Woods Hole, Massachusetts 02543

Nachmansohn, Dr. David, Department of Neurology, Columbia University, College of Physicians and Surgeons, New York, New York 10032

Narahashi, Dr. Toshio, Department of Physiology, Duke University Medical Center, Durham, North Carolina 27706

Nasatir, Dr. Maimon, Department of Biology, University of Toledo, Toledo, Ohio 43606

Nason, Dr. Alvin, McCollum-Pratt Institute, The Johns Hopkins University, Baltimore, Maryland 21218

Nelson, Dr. Leonard, Department of Physiology, Medical College of Ohio at Toledo, Toledo, Ohio 43614

Nicholls, Dr. John Graham, Department of Physiology, Stanford University, Stanford, California 94305

Nicoll, Dr. Paul A., R. R. 12, Box 286, Bloomington, Indiana 47401

NIU, DR. MAN-CHIANG, Department of Biology, Temple University, Philadelphia, Pennsylvania 19122

NOE, DR. BRYAN D., Department of Anatomy, Emory University, Atlanta, Georgia 30345

NOVIKOFF, DR. ALEX B., Department of Pathology, Albert Einstein College of Medicine, Bronx, New York 10461

NYSTROM, DR. RICHARD A., Hudson Valley Community College, 80 Vandenburgh Ave., Troy, New York 12180

OCHOA, DR. SEVERO, 530 East 72nd Street, New York, New York 10021

ODUM, DR. EUGENE, Department of Zoology, University of Georgia, Athens, Georgia 30601

OLSON, DR. JOHN M., Department of Biology, Brookhaven National Laboratory, Upton, New York 11973

OSCHMAN, DR. JAMES L., Department of Biological Sciences, Northwestern University, Evanston, Illinois 60201

PALMER, DR. JOHN D., Department of Zoology, University of Massachusetts, Amherst, Massachusetts 01002

PALTI, DR. YORAM, Head, Department of Physiology and Biophysics, Israel Institute of Technology, 12 Haaliya St., Bat-Galim, P. O. B. 9649, Haifa, Israel

PAPPAS, DR. GEORGE D., Department of Neuroscience, Albert Einstein College of Medicine, Bronx, New York 10461

PEARLMAN, DR. ALAN L., Department of Physiology, Washington University School of Medicine, St. Louis, Missouri 63110

PERKINS, DR. C. D., 621 Lake Drive, Princeton, New Jersey 08540

PERSON, DR. PHILIP, Special Dental Research Program, Veterans Administration Hospital, Brooklyn, New York 11219

PETTIBONE, DR. MARIAN H., Division of Marine Invertebrates, U. S. National Museum, Washington, D. C. 20025

PFOHL, DR. RONALD J., Department of Zoology, Miami University, Oxford, Ohio 45056

PHILPOTT, DR. DELBERT E., MASA Ames Research Center, Moffett Field, California 94035

PIERCE, DR. SIDNEY K., JR., Department of Zoology, University of Maryland, College Park, Maryland 20740

PINTO, DR. LAWRENCE, Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907

POLLARD, DR. HARVEY B., National Institutes of Health, F. Bldg. 10, Rm. 10B17, Bethesda, Maryland 20014

POLLARD, DR. THOMAS D., Department of Anatomy, Harvard Medical School, Boston, Massachusetts 02115

POLLOCK, DR. LELAND W., Department of Zoology, Drew University, Madison, New Jersey 07940

PORTER, DR. KEITH R., 748 11th Street, Boulder, Colorado 80302

POTTER, DR. DAVID, Department of Neurophysiology, Harvard Medical School, Boston, Massachusetts 02115

POTTS, DR. WILLIAM T. W., Department of Biology, University of Lancaster, Lancaster, England, U. K.

PRENDERGAST, DR. ROBERT A., Department of Pathology and Ophthalmology, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

PRICE, DR. CARL A., Wakeman Institute of Microbiology, Rutgers University, New Brunswick, New Jersey 08903

PROSSER, DR. C. LADD, Department of Physiology and Biophysics, Burrill Hall 524, University of Illinois, Urbana, Illinois 61801

PROVASOLI, DR. LUIGI, Haskins Laboratories, 165 Prospect Street, New Haven, Connecticut 06520

PRUSCH, DR. ROBERT D., Division of Biomedical Sciences, Brown University, Providence, Rhode Island 02904

PRYTZ, DR. MARGARET MCDONALD, 21 McCouns Lane, Oyster Bay, New York 11771

PRZYBYLSKI, DR. RONALD J., Department of Anatomy, Case Western Reserve University, Cleveland, Ohio 44101

QUATRANO, DR. RALPH S., Department of Botany, Oregon State University, Corvallis, Oregon 97330

RABIN, DR. HARVEY, Director, Department of Virology and Cell Biology, Bionetics Research Laboratories, 5510 Nicholson Lane, Kensington, Maryland 20795

RANKIN, DR. JOHN S., Department of Zoology, University of Connecticut, Storrs, Connecticut 06268

RANZI, DR. SILVIO, Department of Zoology, University of Milan, Via Celonia 10, Milan, Italy

RATNER, DR. SARAH, Department of Biochemistry, The Public Health Research Institute of the City of New York, Inc., 455 First Avenue, New York, New York 10016

REBHUN, DR. LIONEL I., Department of Biology, Gilmer Hall, University of Virginia, Charlottesville, Virginia 22901

REDDAN, DR. JOHN R., Department of Biological Sciences, Oakland University, Rochester, Michigan 48063

REDFIELD, DR. ALFRED C., Maury Lane, Woods Hole, Massachusetts 02543

REINER, DR. JOHN M., Department of Biochemistry, Albany Medical College of Union University, Albany, New York 12208

RENN, DR. CHARLES E., Route 2, Hampstead, Maryland 21074

REUBEN, DR. JOHN P., Department of Neurology, Columbia University, College of Physicians and Surgeons, New York, New York 10032

REYNOLDS, DR. GEORGE THOMAS, Department of Physics, Princeton University, Princeton, New Jersey 08540

REZNIKOFF, DR. PAUL, 151 Sparks Ave., Pelham, New York 10803

RICE, DR. ROBERT VERNON, Mellon Institute, Carnegie-Mellon University, 4400 Fifth Avenue, Pittsburgh, Pennsylvania 15213

RICH, DR. ALEXANDER, Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

RICHARDS, DR. OSCAR W., Pacific University, College of Optometry, Forrest Grove, Oregon 97116

RIPPS, DR. HARRIS, Department of Opthalmology, New York University, School of Medicine, 550 1st Avenue, New York, New York 10016

ROBERTS, DR. JOHN L., Department of Zoology, University of Massachusetts, Amherst, Massachusetts 01002

ROBINSON, DR. DENIS M., 19 Orlando Avenue, Arlington, Massachusetts 02174

ROCKSTEIN, DR. MORRIS, Department of Physiology, University of Miami School of Medicine, P. O. Box 975 Biscayne Annex, Miami, Florida 33152

RONKIN, DR. RAPHAEL E., 3212 McKinley St., N. W., Washington, D. C. 20015

ROSE, DR. BIRGIT, Department of Physiology, University of Miami School of Medicine, Miami, Florida 33152

ROSE, DR. S. MERYL, 34 High St., Woods Hole, Massachusetts 02543

ROSENBAUM, DR. JOEL L., Kline Biology Tower, Yale University, New Haven, Connecticut 06510

ROSENBERG, DR. EVELYN K., Jersey City State College, Jersey City, New Jersey 07305

ROSENBERG, DR. PHILLIP, Division of Pharmacology, University of Connecticut, School of Pharmacy, Storrs, Connecticut 06268

ROSENBLUTH, DR. JACK, Department of Physiology, New York University, School of Medicine, 550 First Avenue, New York, New York 10016

ROSENBLUTH, RAJA, #10, 3250 West 4th Avenue, Vancouver 8, British Columbia, Canada V6K IR9

ROSENKRANZ, DR. HERBERT S., Department of Microbiology, Columbia University, College of Physicians and Surgeons, 630 West 168th St., New York, New York 10032

ROSENTHAL, DR. THEODORE B., Department of Anatomy, University of Pittsburgh Medical School, Pittsburgh, Pennsylvania 15213

ROSLANSKY, DR. JOHN, 26 Albatross, Woods Hole, Massachusetts 02543

ROTH, DR. JAY S., Division of Biological Sciences, Section of Biochemistry and Biophysics, University of Connecticut, Storrs, Connecticut 06268

ROWLAND, DR. LEWIS P., Department of Neurology, Columbia University, College of Physicians and Surgeons, 630 W. 168th St., New York, New York 10032

RUBINOW, DR. SOL I., Cornell University, Medical College, Department of Biomathematics, New York, New York 10012

RUSHFORTH, DR. NORMAN B., Department of Biology, Case Western Reserve University, Cleveland, Ohio 44106

RUSSELL, DR. JOHN M., Department of Biophysics, University of Texas, Medical Branch, Galveston, Texas 77550

RUSSELL-HUNTER, DR. W. D., Department of Biology, Lyman Hall, Syracuse University, Syracuse, New York 13210

RUSTAD, DR. RONALD C., Department of Radiology, Case Western Reserve University, Cleveland, Ohio 44106

RUTMAN, DR. ROBERT J., University of Pennsylvania, School of Veterinary Medicine, Department of Animal Biology, 3800 Spruce Street, Philadelphia, Pennsylvania 19104

RYTHER, DR. JOHN H., Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543

SAGER, DR. RUTH, Sidney Farber Cancer Center, 35 Binney St., Boston, Massachusetts 02115

SALMON, DR. EDWARD D., Marine Biological Laboratory, Woods Hole, Massachusetts 02543

SALZBERG, DR. BRIAN M., Department of Physiology, University of Pennsylvania, 4010 Locust St., Philadelphia, Pennsylvania 19174

SANBORN, DR. RICHARD C., Dean, Purdue University Regional Campus, 1125 East 38th Street, Indianapolis, Indiana 46205

SANDERS, DR. HOWARD L., Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543

SATO, DR. HIDEMI, 217 Leidy Building, Department of Biology, University of Pennsylvania, Philadelphia, Pennsylvania 19104

SAUNDERS, DR. JOHN W., JR., Department of Biological Sciences, State University of New York at Albany, Albany, New York 12203

SAZ, DR. ARTHUR KENNETH, Department of Microbiology, Georgetown University Medical and Dental Schools, 3900 Reservoir Road, Washington, D. C. 20051

SCHACHMAN, DR. HOWARD K., Department of Biochemistry, University of California, Berkeley, California 94720

SCHARRER, DR. BERTA V., Department of Anatomy, Albert Einstein College of Medicine, 1300 Morris Parkway, New York, New York 10461

SCHIFF, DR. JEROME A., Department of Biology, Brandeis University, Waltham, Massachusetts 02154

SCHLESINGER, DR. R. WALTER, Department of Microbiology, Rutgers Medical School, P. O. Box 101, Piscataway, New Jersey 08854

SCHMEER, SISTER ARLINE CATHERINE, O.P., The American Medical Center of Denver, 6401 W. Colfax Ave., Denver, Colorado 80214

SCHNEIDERMAN, DR. HOWARD A., Center for Pathobiology, School of Biological Sciences, University of California, Irvine, California 92717

SCHOLANDER, DR. P. F., Scripps Institution of Oceanography, La Jolla, California 92037

SCHOPF, DR. THOMAS J. M., Department of the Geophysical Sciences, University of Chicago, 5734 S. Ellis Avenue, Chicago, Illinois 60637

SCHOTTÉ, DR. OSCAR E., Department of Biology, Amherst College, Amherst, Massachusetts 01002

SCHUEL, DR. HERBERT, Department of Biochemistry, State University of New York, Downstate Medical Center, 450 Clarkson Ave., Brooklyn, New York 11203

SCHUETZ, DR. ALLEN WALTER, The Johns Hopkins University School of Hygiene and Public Health, Baltimore, Maryland 21205

SCHWARTZ, DR. TOBIAS L., Biological Sciences Group, University of Connecticut, Storrs, Connecticut 06268

SCOTT, DR. ALAN C., Colby College, Waterville, Maine 04901

SCOTT, DR. GEORGE T., Department of Biology, Oberlin College, Oberlin, Ohio 44074

SEARS, DR. MARY, Box 152, Woods Hole, Massachusetts 02543

SEGAL, DR. SHELDON J., Population Council, The Rockfeller University, New York, New York 10021

SELIGER, DR. HOWARD H., McCollum-Pratt Institute, The Johns Hopkins University, Baltimore, Maryland 21218

Selman, Dr. Kelly, Division of Anatomy, Department of Pathology, University of Florida, Gainesville, Florida 32601

Senft, Dr. Joseph P., Department of Biology, Juniata College, Huntingdon, Pennsylvania 16652

Shanklin, Dr. Douglas R., P. O. Box 1267, Gainesville, Florida 32602

Shapiro, Dr. Herbert, 6025 North 13th Street, Philadelphia, Pennsylvania 19141

Shaver, Dr. John R., Department of Zoology, Michigan State University, East Lansing, Michigan 48823

Shedlovsky, Dr. Theodore, The Rockefeller University, 66th Street and York Ave., New York, New York 10021

Shemin, Dr. David, Department of Chemistry and Biological Sciences, Northwestern University, Evanston, Illinois 60201

Shephard, Dr. David C., P.O. Box 44, Woods Hole, Massachusetts 02543

Shepro, Dr. David, Department of Biology, Boston University, 2 Cummington Street, Boston, Massachusetts 02215

Sherman, Dr. I. W., Division of Life Sciences, University of California, Riverside, California 92502

Shilo, Dr. Moshe, Head, Department of Microbiological Chemistry, Hebrew University, Jersusalem, Israel

Sichel, Dr. Elsa Keil, Emeritus Professor of Biology, Trinity College, Burlington, Vermont 05401

Siegel, Dr. Irwin M., Department of Ophthalmology, New York University Medical Center, 550 First Avenue, New York, New York 10016

Siegelman, Dr. Harold W., Department of Biology, Brookhaven National Laboratory, Upton, New York 11973

Silva, Dr. Paul C., Department of Botany, University of California, Berkeley, California 94704

Simmons, Dr. John E., Jr., Department of Biology, University of California, Berkeley, California 94704

Simon, Dr. Eric J., New York University Medical School, 550 First Avenue, New York, New York 10016

Sjodin, Dr. Raymond A., Department of Biophysics, University of Maryland School of Medicine, Baltimore, Maryland 21201

Skinner, Dr. Dorothy M., Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830

Slobodkin, Dr. Lawrence B., Department of Biology, State University of New York, Stony Brook, Long Island, New York 11790

Smith, Homer P., General Manager, Marine Biological Laboratory, Woods Hole, Massachusetts 02543

Smith, Paul Ferris, Church Street, Woods Hole, Massachusetts 02543

Smith, Dr. Ralph I., Department of Zoology, University of California, Berkeley, California 94720

Sonneborn, Dr. T. M., Department of Zoology, Indiana University, Bloomington, Indiana 47401

Sonnenblick, Dr. B. P., Department of Biology, Rutgers University, 195 University Avenue, Newark, New Jersey 07102

Sorenson, Dr. Albert L., Department of Biology, Brooklyn College, Brooklyn, New York 11210

Sorenson, Dr. Martha M., Department of Neurology, Columbia University, College of Physicians and Surgeons, New York, New York 10032

Spector, Dr. A., Black Bldg., Rm. 1516, Columbia University, College of Physicians and Surgeons, New York, New York 10032

Spiegel, Dr. Evelyn, Research Associate, Dartmouth College, Hanover, New Hampshire 03755

Spiegel, Dr. Melvin, Department of Biological Sciences, Dartmouth College, Hanover, New Hampshire 03755

Spindel, Dr. William, Division of Chemistry and Chemical Technology, National Academy of Sciences, 2101 Constitution Ave., Washington, D. C. 20418

Spira, Dr. Micha E., Department of Zoology, Hebrew University, Jerusalem, Israel

Spirtes, Dr. Morris Albert, Veterans Administration Hospital, 1601 Perdido Street, New Orleans, Louisiana 70112

Spray, Dr. David C., Department of Neuroscience, Albert Einstein College of Medicine, Bronx, New York 10461

Starr, Dr. Richard C., Department of Botany, Indiana University, Bloomington, Indiana 47401

Starzak, Dr. Michael E., Department of Chemistry, State University of New York, Binghamton, New York 13901

Steinbach, Dr. H. Burr, Oceanic Foundation, Makapuu Point, Waimanalo, Hawaii 96795

Steinberg, Dr. Malcolm S., Department of Biology, Princeton University, Princeton, New Jersey 08540

Steinhardt, Dr. Jacinto, 306 Reiss Bldg., Georgetown University, Washington, D. C. 20007

Stephens, Dr. Grover C., Division of Biological Sciences, University of California, Irvine, California 92650

Stephens, Dr. Raymond E., Department of Biology, Brandeis University, Waltham, Massachusetts 02154

Stetten, Dr. Majorie R., National Institutes of Health, Bldg. 10, 9B-02, Bethesda, Maryland 20014

Stokes, Dr. Darrell R., Gaijinkenkyuinto–Shukusha, Igakubu-Konai Kyudai, Maidashi, 3 Chome 1-1, Higashiku, Fukuoka Shi, Japan 812

Stracher, Alfred, Downstate Medical Center, State University of New York at Brooklyn, 450 Clarkson Avenue, Brooklyn, New York 11203

Strehler, Dr. Bernard L., 1 Laguna Circle Drive, Agoura, California 91307

Strittmatter, Dr. Philipp, Department of Biochemistry, University of Connecticut School of Medicine, Health Center, Hartford, Connecticut 06105

Stuart, Dr. Ann E., Department of Neurobiology, Harvard Medical School, 25 Shattuck St., Boston, Massachusetts 02115

Sulkin, Dr., S. Edward, Department of Bacteriology, Southwestern Medical School, University of Texas, Dallas, Texas 75221

Summers, Dr. William C., Huxley College, Western Washington State University, Bellingham, Washington 98225

SUSSMAN, DR. MAURICE, Department of Life Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania 15260

SWANSON, DR. CARL PONTIUS, Department of Botany, University of Massachusetts, Amherst, Massachusetts 01002

SWOPE, GERARD, JR., Blinn Road, Box 345, Croton-on-Hudson, New York 10520

SZABÓ, DR. GEORGE, Harvard School of Dental Medicine, 188 Longwood Avenue, Boston, Massachusetts 02115

SZAMIER, DR. ROBERT BRUCE, Harvard Medical School, Berman Gund Laboratory, Eye and Ear Infirmary, 243 Charles Street, Boston, Massachusetts 02114

SZENT-GYÖRGYI, DR. ALBERT, Institute for Muscle Research, Marine Biological Laboratory, Woods Hole, Massachusetts 02543

SZENT-GYÖRGYI, DR. ANDREW G., Department of Biology, Brandeis University, Waltham, Massachusetts 02154

TAKASHIMA, DR. SHIRO, Department of Bioengineering, University of Pennsylvania, Philadelphia, Pennsylvania 19104

TANZER, DR. MARVIN L., Department of Biochemistry, University of Connecticut, School of Medicine, Farmington, Connecticut 06032

TASAKI, DR. ICHIJI, Laboratory of Neurobiology, National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland 20014

TAYLOR, DR. DOUGLASS L., The Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138

TAYLOR, DR. ROBERT E., Laboratory of Biophysics, National Institute of Neurological Diseases and Blindness, National Institutes of Health, Bethesda, Maryland 20014

TAYLOR, DR. W. ROWLAND, 1540 Northbourne Rd., Baltimore, Maryland 21239

TELFER, DR. WILLIAM H., Department of Biology, University of Pennsylvania, Philadelphia, Pennsylvania 19104

deTERRA, DR. NOEL, Department of Anatomy, Hahnemann Medical College, 230 N. Broad St., Philadelphia, Pennsylvania 19102

THALER, DR. M. MICHAEL, University of California, San Francisco, California 94106

TIFFNEY, DR. WESLEY N., 226 Edge Hill Rd., Sharon, Massachusetts 02067

TRAGER, DR. WILLIAM, The Rockefeller University, 66th Street and York Avenue, New York, New York 10021

TRAVIS, DR. D. M., Department of Pharmacology, University of Florida, Gainesville, Florida 32601

TRAVIS, DR. DOROTHY FRANCES, Department of Pharmacology, University of Florida, Gainesville, Florida 32601

TRINKAUS, DR. J. PHILIP, Department of Biology, Yale University, New Haven, Connecticut 06510

TROLL, DR. WALTER, Department of Environmental Medicine, New York University, College of Medicine, New York, New York 10016

TWEEDELL, DR. KENYON S., 210 E. Bartlett Street, South Bend, Indiana 46601

URETZ, DR. ROBERT B., Department of Biophysics, University of Chicago, Chicago, Illinois 60637

VALIELA, DR. IVAN, Boston University Marine Program, Marine Biological Laboratory, Woods Hole, Massachusetts 02543

VALOIS, JOHN, Marine Biological Laboratory, Woods Hole, Massachusetts 02543

Van Holde, Dr. Kensal Edward, Oregon State University, Department of Biochemistry and Biophysics, Corvallis, Oregon 97331

Villee, Dr. Claude A., Department of Biochemistry, Harvard Medical School, Boston, Massachusetts 02115

Vincent, Dr. Walter S., Department of Biology, University of Delaware, Newark, Delaware 19711

Wainio, Dr. W. W., Bureau of Biological Research, Rutgers University, New Brunswick, New Jersey 08901

Waksman, Dr. Bryon, Department of Microbiology, Yale University, New Haven, Connecticut 06510

Wald, Dr. George, The Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138

Walker, Dr. Charles A., Department of Physiology and Pharmacology, School of Veterinary Medicine, Tuskegee Institute, Tuskegee, Alabama, 36088

Wall, Dr. Betty J., Department of Biological Sciences, Northwestern University, Evanston, Illinois 60201

Wallace, Dr. Robin A., P. O. Box Y, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37890

Wang, Dr. A., Bedford Road, Lincoln Massachusetts 01773

Warner, Dr. Robert C., Department of Molecular and Cell Biology, University of California, Irvine, California 92664

Warren, Dr. Leonard, Department of Therapeutic Research, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104

Waterman, Dr. T. H., 610 Kline Biology Tower, Yale University, New Haven, Connecticut 06520

Watkins, Dr. Dudley Taylor, Department of Anatomy, University of Connecticut, Farmington, Connecticut 06032

Watson, Dr. Stanley Wayne, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543

Webb, Dr. H. Marguerite, Department of Biological Sciences, Goucher College, Towson, Maryland 21204

Weber, Dr. Annemarie, Department of Biochemistry, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104

Webster, Dr. Ferris, Associate Director for Research, Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543

Weidner, Dr. Earl, Department of Zoology, Louisiana State University, Baton Rouge, Louisiana 70803

Weisenberg, Dr. Richard, Department of Biology, Temple University, Philadelphia, Pennsylvania 19104

Weiss, Dr. Leon P., Department of Anatomy, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Weissmann, Dr. Gerald, Professor of Medicine, New York University, 550 First Avenue, New York, New York 10016

Werman, Dr. Robert, Department of Zoology, Hebrew University, Jerusalem, Israel

Whiting, Dr. Anna R., Woods Hole, Massachusetts 02543

Whiting, Dr. Phineas, Woods Hole, Massachusetts 02543

Whittaker, Dr. J. Richard, Wistar Institute of Anatomy and Biology, 36th Street at Spruce, Philadelphia, Pennsylvania 19104

Wiercinski, Dr. Floyd J., Department of Biology, Northeastern Illinois University, 5500 North St. Louis Avenue, Chicago, Illinois 60625

Wigley, Dr. Roland L., National Marine Fisheries Service, Woods Hole, Massachusetts 02543

Wilbur, Dr. C. G., Chairman, Department of Zoology, Colorado State University, Fort Collins, Colorado 80521

Wilson, Dr. Darcy B., Department of Pathology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104

Wilson, Dr. Edward O., Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts 02138

Wilson, Dr. T. Hastings, Department of Physiology, Harvard Medical School, Boston, Massachusetts 02115

Wilson, Dr. Walter L., Department of Biology, Oakland University, Rochester, Michigan 48063

Winters, Dr. Robert Wayne, Department of Pediatrics, Columbia University, College of Physicians and Surgeons, New York, New York 10032

Witkovsky, Dr. Paul, Department of Opthalmology, Columbia University, 630 West 168th Street, New York, New York 10032

Wittenberg, Dr. Jonathan B., Department of Physiology and Biochemistry, Albert Einstein College of Medicine, New York, New York 10461

Wyse, Dr. Gordon A., Department of Zoology, University of Massachusetts, Amherst, Massachusetts 01002

Wyttenbach, Dr. Charles R., Department of Physiology and Cell Biology, University of Kansas, Lawrence, Kansas 66044

Yntema, Dr. C. L., Department of Anatomy, State University of New York, Upstate Medical Center, Syracuse, New York 13210

Young Dr. David K., Fort Pierce Bureau, Smithsonian Institution, RFD#1, Box 194-C, Fort Pierce, Florida 33450

Yphantis, Dr. David A., Department of Biochemistry and Biophysics, University of Connecticut, Storrs, Connecticut 06268

Zigman, Dr. Seymour, University of Rochester School of Medicine and Dentistry, 260 Crittenden Boulevard, Rochester, New York 14620

Zimmerman, Dr. A. M., Department of Zoology, University of Toronto, Toronto 5, Ontario, Canada

Zinn, Dr. Donald J., P. O. Box 589, Falmouth, Massachusetts 02540

Zorzoli, Dr. Anita, Department of Biology, Vassar College, Poughkeepsie, New York 12601

ASSOCIATE MEMBERS

Abelson, Dr. and Mrs. Philip H.
Acheson, Dr. and Mrs. George
Ackroyd, Dr. and Mrs. Frederick W.
Adelberg, Dr. and Mrs. Edward A.
Adelman, Dr. and Mrs. William J.
Allen, Miss Camilla K.
Allen, Mrs. A. D.
Amberson, Mrs. William R.
Andrews, Mr. William R.
Armstrong, Mrs. Philip B.
Armstrong, Dr. and Mrs. Samuel C.

Arnold, Dr. and Mrs. John
Atwood, Dr. and Mrs. Kimball C.
Bacon, Mr. Robert
Ball, Dr. and Mrs. Eric G.
Ballantine, Dr. and Mrs. H. T., Jr.
Banks, Mr. and Mrs. W. L.
Barrows, Mrs. Albert W.
Bartow, Mrs. Clarence W.
Bartow, Mrs. Philip K.
Bennett, Dr. and Mrs. Michael V. L.
Bernheimer, Dr. Alan W.
Blackburn, Dr. and Mrs. George L.
Boettiger, Dr. and Mrs. Edward G.
Borgese, Mrs. Thomas A.
Bowles, Mr. and Mrs. Francis P.
Bradley, Dr. and Mrs. Charles C.
Bronson, Mr. and Mrs. Samuel C.
Brown, Dr. and Mrs. Dugald E. S.
Brown, Dr. and Mrs. F. A., Jr.
Brown, Dr. and Mrs. Thornton
Buck, Mrs. John B.
Buffington, Mrs. Alice H.
Buffington, Mrs. George
Bunting, Dr. Mary I.
Burdick, Dr. C. Lalor
Burrough, Mrs. Arnold H.
Burt, Mr. and Mrs. Charles E.
Busser, Dr. and Mrs. John H.
Butler, Mrs. E. G.
Calkins, Mr. and Mrs. G. N., Jr.
Campbell, Mr. and Mrs. Worthington, Jr.
Carlton, Mr. and Mrs. Winslow G.
Carpenter, Mr. Donald F.
Cashman, Mr. and Mrs. Eugene R.
Chambers, Dr. and Mrs. Edward L.
Cheney, Dr. Ralph H.
Christman, Dr. and Mrs. George D.
Clark, Dr. and Mrs. Arnold M.
Clark, Mr. and Mrs. Hays
Clark, Mrs. James McC.
Clark, Dr. and Mrs. Leonard B.
Clark, Mrs. LeRoy
Clark, Mr. and Mrs. W. Van Alan
Clement, Dr. and Mrs. A. C.
Clements, Mr. and Mrs. David T.
Clowes, Mr. Allen W.
Clowes, Dr. and Mrs. G. H. A., Jr.
Cochran, Mr. and Mrs. F. Morris
Cohen, Dr. and Mrs. Seymour
Connell, Mr. and Mrs. W. J.
Copeland, Mrs. D. Eugene
Copeland, Mr. and Mrs. Preston S.
Costello, Mrs. Donald P.
Cramer, Mr. and Mrs. Ian D. W.
Crane, Mr. John
Crane, Josephine, Foundation
Crane, Miss Louise
Crane, Mrs. W. Carey
Cross, Mr. and Mrs. Norman C.
Crossley, Mr. and Mrs. Archibald M.
Crowell, Dr. and Mrs. Sears
Curtis, Dr. and Mrs. W. D.
Daignault, Mr. and Mrs. A. T.
Daniels, Mr. and Mrs. Bruce G.
Daniels, Mrs. F. Harold
Day, Mr. and Mrs. Pomeroy
Draper, Mrs. Mary C.
DuBois, Dr. and Mrs. A. B.
DuPont, Mr. A. Felix, Jr.
Dyer, Mr. and Mrs. Arnold
Eastman, Mr. and Mrs. Charles E.
Ebert, Dr. and Mrs. James D.
Egloff, Dr. and Mrs. F. R. L.
Elliott, Mrs. Alfred M.
Elsmith, Mrs. Dorothy O.
Epel, Mrs. David
Evans, Mr. and Mrs. Dudley
Ewing, Dr. and Mrs. Gifford C.
Fenno, Mrs. Edward N.
Ferguson, Dr. and Mrs. J. J., Jr.
Fine, Dr. and Mrs. Jacob
Firestone, Mr. and Mrs. Edwin
Fisher, Mr. Frederick S., III
Fisher, Dr. and Mrs. Saul H.
Francis, Mr. and Mrs. Lewis W., Jr.
Fries, Mr. and Mrs. E. F. B.
Fye, Dr. and Mrs. Paul M.
Gabriel, Dr. and Mrs. Mordecai L.
Gaiser, Dr. and Mrs. David W.
Galtsoff, Dr. and Mrs. Paul S.
Gamble, Mrs. Richard B.
Garfield, Miss Eleanor
Garrey, Dr. and Mrs. Walter

Gellis, Dr. and Mrs. Sydney
German, Dr. and Mrs. James L., III
Gifford, Mr. and Mrs. John A.
Gifford, Dr. and Mrs. Prosser
Gilbert, Dr. and Mrs. Daniel L.
Gilchrist, Mrs. John M.
Gildea, Dr. Margaret C. L.
Gillette, Mr. and Mrs. Robert S.
Glass, Dr. and Mrs. H. Bentley
Glazebrook, Mrs. James R.
Glusman, Dr. and Mrs. Murray
Goldman, Dr. and Mrs. Allen S.
Goldring, Dr. Irene P.
Goldstein, Mrs. Moise H., Jr.
Graham, Dr. and Mrs. Herbert W.
Grant, Dr. and Mrs. Philip
Grassle, Mr. and Mrs. J. K.
Green, Miss Gladys M.
Greene, Mr. and Mrs. William C.
Greer, Mr. and Mrs. W. H., Jr.
Greif, Dr. and Mrs. Roger L.
Grosch, Dr. and Mrs. Daniel S.
Gruson, Mr. and Mrs. Edward
Gunning, Mr. and Mrs. Robert
Hancox, Capt. and Mrs. Frederick
Handler, Dr. and Mrs. Philip
Hanna, Mr. and Mrs. Thomas C.
Harrington, Mr. and Mrs. R. D.
Harvey, Dr. and Mrs. Edmund N., Jr.
Harvey, Dr. and Mrs. Richard B.
Haskins, Mrs. Caryl P.
Hassett, Dr. and Mrs. Charles
Hastings, Mrs. J. Woodland
Heffron, Dr. and Mrs. Roderick
Hellman, Mr. John R.
Henley, Dr. Catherine
Hervey, Dr. and Mrs. John P.
Hiam, Mr. and Mrs. E. W.
Hiatt, Dr. and Mrs. Howard
Hibbard, Miss Hope
Hill, Mrs. Samuel E.
Hirschfeld, Mrs. Nathan B.
Hocker, Mr. and Mrs. Lon
Hopkins, Mrs. Hoyt S.
Horwitz, Dr. and Mrs. Norman H.
Hough, Mr. and Mrs. George A., Jr.
Hough, Mr. and Mrs. John T.
Houston, Mr. and Mrs. Howard E.
Huettner, Dr. and Mrs. Robert
Hunziker, Mr. and Mrs. Herbert E.
Hurwitch, Mr. and Mrs. Leo H.
Inoué, Mrs. Shinya
Ireland, Mrs. Herbert A.
Issokson, Mr. and Mrs. Israel
Janney, Mr. and Mrs. Wistar
Jewett, Mr. and Mrs. G. F., Jr.
Jones, Mr. and Mrs. Dewitt, III
Jordan, Dr. and Mrs. Edwin P.
Kaan, Dr. Helen W.
Kahler, Mr. and Mrs. George A.
Kahler, Mrs. Robert W.
Kaighn, Dr. and Mrs. Morris E.
Keith, Mr. Jean R.
Kennedy, Dr. and Mrs. Eugene P.
Kenefick, Mrs. Theodore G.
Keosian, Mrs. Jessie
Kingwell, The Rev. and Mrs. Wilbur J.
Kinnard, Mr. and Mrs. L. R.
Kohn, Dr. and Mrs. Henry I.
Koller, Dr. and Mrs. Lewis R.
Kris, Dr. and Mrs. Anton O.
Lash, Dr. and Mrs. Jay
Laster, Dr. and Mrs. Leonard
Lawrence, Mr. and Mrs. Frederick V.
Lawrence, Mrs. William
Lazarow, Mrs. Arnold
Leister, Mr. and Mrs. Robert E.
Lemann, Mrs. Lucy B.
Lenher, Mr. and Mrs. Samuel
Levine, Dr. and Mrs. Rachmiel
Levy, Dr. and Mrs. Milton
Lillie, Mrs. Karl C.
Lilly, Mr. and Mrs. Josiah K.
Lobb, Prof. and Mrs. John
Loeb, Mrs. Robert F.
Long, Mrs. G. C.
Lorand, Mrs. Lazlo
Lovell, Mr. and Mrs. Hollis R.
Lowengard, Mrs. Joseph
Luria, Dr. and Mrs. S. E.
Mackey, Mr. and Mrs. William K.
MacLeish, Mr. and Mrs. William
MacNary, Mr. B. Glenn

MacNichol, Dr. and Mrs. Edward F., Jr.
Marks, Dr. and Mrs. Paul A.
Marsland, Dr. and Mrs. Douglas
Martyna, Mr. and Mrs. Joseph
Marvin, Dr. Dorothy H.
Mather, Mr. and Mrs. Frank J., III
Mavor, Mrs. James W., Sr.
McCusker, Mr. and Mrs. Paul T.
McElroy, Mrs. Nella W.
McGillicuddy, Dr. and Mrs. J. J.
McKenzie, Mrs. Kenneth C.
McLane, Mrs. T. Thorne
McLardy, Dr. and Mrs. Turner
Meigs, Mr. and Mrs. Arthur
Meigs, Dr. and Mrs. J. Wister
Meissner, Mr. John Harper
The Mellon Foundation
Metz, Mrs. Charles B.
Meyers, Mr. and Mrs. Richard
Mixter, Mr. and Mrs. W. J., Jr.
Moloney, Dr. Albert M.
Montgomery, Dr. and Mrs. Charles H.
Morse, Mr. and Mrs. Charles L., Jr.
Morse, Mr. and Mrs. Richard S.
Moses, Mr. and Mrs. George L.
Moul, Mrs. Edwin T.
Neuberger, Mrs. Harry H.
Newton, Miss Helen K.
Nichols, Mrs. George
Nickerson, Mr. and Mrs. Frank L.
Norman, Mr. and Mrs. Andrew E.
Olmstead, Mr. and Mrs. Christopher
O'Neil, Mr. Thomas N.
Ortins, Mr. Armand
Park, Mr. and Mrs. Malcolm S.
Park, Mr. and Mrs. Franklin A.
Parmentier, Mr. George L.
Patten, Mrs. Bradley M.
Pendergast, Mrs. Claudia
Pendleton, Dr. Murray E.
Pennington, Miss Anne H.
Perkins, Mr. and Mrs. Courtland D.
Person, Dr. and Mrs. Philip
Peterson, Mr. and Mrs. E. Gunnar
Philippe, Mr. and Mrs. Pierre
Porter, Dr. and Mrs. Keith R.
Prosser, Mrs. C. Ladd
Putnam, Mr. and Mrs. W. A., III
Ratcliffe, Mr. Thomas G., Jr.
Raymond, Dr. and Mrs. Samuel
Read, Mrs. Clark
Redfield, Dr. and Mrs. Alfred C.
Renek, Mr. and Mrs. Morris
Reynolds, Dr. and Mrs. George
Reznikoff, Dr. and Mrs. Paul
Riggs, Mr. and Mrs. Lawrason, III
Riina, Mr. and Mrs John R.
Robb, Ms. Alison A.
Robertson, Dr. and Mrs. C. W.
Robinson, Dr. and Mrs. Denis M.
Rogers, Mrs. Julian
Root, Mrs. Walter S.
Ross, Mrs. John
Rowe, Mrs. William S.
Rugh, Dr. and Mrs. Roberts
Russell, Mr. and Mrs. Henry D.
Ryder, Mr. and Mrs. Francis D.
Saunders, Dr. and Mrs. John W.
Saunders, Mrs. Lawrence
Savery, Mr. Roger
Sawyer, Mr. and Mrs. John E.
Schlesinger, Mrs. R. Walter
Scott, Mrs. George T.
Scott, Mrs. Norman
Segal, Dr. and Mrs. Sheldon
Shapiro, Mrs. Howard E.
Shemin, Dr. and Mrs. David
Shepro, Dr. and Mrs. David
Sherman, Dr. and Mrs. Irwin
Smith, Mrs. Homer P.
Smith, Mr. VanDorn C.
Speidel, Dr. and Mrs. Carl C.
Steinbach, Dr. and Mrs. H. B.
Stetten, Dr. and Mrs. Dewitt, Jr.
Stunkard, Dr. Horace
Sturtevant, Mrs. P.
Swanson, Dr. and Mrs. Carl P.
Swope, Mr. and Mrs. Gerard L.
Swope, Mr. and Mrs. Gerard, Jr.
Swope, Miss Henriette H.
Tanner, Dr. and Mrs. Harvey A.
Tartakoff, Dr. Helen

Taylor. Dr. and Mrs. W. Randolph
Tietje, Mr. and Mrs. Emil D.
Todd, Mr. and Mrs. Gordon F.
Tolkan, Mr. and Mrs. Norman N.
Tompkins, Mrs. B. A.
Trager, Mrs. William
Troll, Dr. and Mrs. Walter
Tully, Mr. and Mrs. Gordon F.
Valois, Mr. and Mrs. John
Veeder, Mrs. Ronald A.
Vincent, Mrs. Walter S.
Waksman, Dr. and Mrs Bryon H.
Ware, Mr. and Mrs. J. Lindsay
Warren, Dr. and Mrs. Shields
Watt, Mr. and Mrs. John B.
Weisberg, Mr. and Mrs. alfred M.
Wexler, Mr. and Mrs. Robert H.
Wheatley, Dr. Marjorie A.
Wheeler, Dr. and Mrs. Paul S.
Wheeler, Dr. and Mrs. Ralph E.
Whiteley, Mr. and Mrs. G. C., Jr.
Whitney, Mr. and Mrs. Geoffrey G., Jr.
Wickersham, Mr. and Mrs. A. A. Tilney
Wickersham, Mrs. James H., Jr.
Wichterman, Dr. and Mrs. Ralph
Wilber, Dr. and Mrs. Charles G.
Wilhelm, Dr. Hazel S.
Wilson, Mr. and Mrs. Robert E., Jr.
Witmer, Dr. and Mrs. Enos E.
Wolfe, Dr. Charles
Wolfinsohn, Mr. and Mrs. Wolfe
Yntema, Dr. and Mrs. Chester L.
Zinn, Dr. Donald J.
Zwilling, Mrs. Edgar

VI. REPORT OF THE LIBRARIAN

The first Annual Meeting of the East Coast Marine Science Librarians was held at the Marine Biological Laboratory in October. Forty-nine librarians, representing 25 institutions from Nova Scotia to Florida met for two days to discuss areas of mutual cooperation. Everyone attending felt that Woods Hole was a grand place to meet, and the 1976 meeting will probably be held here. The Library never looked better due to the visit of H. M. Hirohito, Emperor of Japan, a few days before the meeting. The reading rooms, catalog room, offices and second floor hall were all repainted and polished for his visit. His Majesty spent over an hour in the catalog room with five scientists and many marine animals.

The Biological Bulletin subscription activities became a library responsibility in the fall. Caki Herrity-Tashiro, former Assistant Editor of The Biological Bulletin, joined the library staff in September, and the Bulletin subscription details involved a third of her time during the year. After a long search by a W.H.O.I. Library Study Committee, Carol Winn joined the Oceanographic staff as Research Librarian in July. In the last six months of the year she added over 700 books and 63 journal titles to our collection. Many were added to expand the material in the Marine Policy section. A number of these new journal titles are shelved in Room 306 rather than in the usual alphabetical order in the regular stack area. This unfortunate arrangement is due to our continuing space problem.

We added 92 new journal titles to the collection and 3,300 bound volumes and books, making a total of 158,864 volumes at the end of 1975.

VII. REPORT OF THE TREASURER

Major progress was made during 1975 in expanding the year-round activities of the Laboratory. This progress is described in the report of the Director. It is also reflected in the operating results for the year which are the subject of the statements which appear below.

In large measure because of the expanded activities, notably the Ecosystems Center, but also because of continuing inflation in all categories of cost, total operating expenditures for 1975 including expenditures under grants administered by the Laboratory amounted to $2,937,494. This was $651,160 or 28.5% greater than the $2,286,334 of similar expenditures in the previous year.

Revenues also increased—from $2,206,598 in 1974 to $2,615,913 in 1975, a favorable variance of $409,315 or 18.5%. The discrepancy in the rate of growth between revenues and expenditures increased the loss from operations to $321,581 in 1975 from $79,736 the previous year.

The increase in operating expenditures reflects a strengthening of the scientific, operating, and financial staff of the Laboratory. The added expense involved was felt necessary to support the new year-round programs and to obtain the financing they require. The success which has already been achieved attests, we believe, to the wisdom of the organizational steps which were taken.

Gifts of $1,019,034 were received in 1975 compared to $657,463 in 1974. Of the 1975 gifts, $968,350 were designated to be used for specific programs. Of this amount, $234,437 was spent in 1975 leaving an unexpended balance of $733,913. In addition, at year end 1975, there remained $887,000 in pledges for specific programs of the Laboratory. These amounts are scheduled to be received in the years 1975 to 1979 inclusive.

It is appropriate to give particular recognition to the generosity of the MBL Associates and other friends of the Laboratory whose gifts of $125,000 made possible the attractive landscaping of the Quadrangle.

The deficit for the year also reflects higher charges than normal for the maintenance and remodeling of the buildings. Such charges should be of lesser magnitude in 1976.

Also adversely affecting 1975 results was the establishment of reserves of $20,000 for doubtful accounts receivable and of $53,841 for the full inventory value of back issues of The Biological Bulletin and of certain books, sales of which are limited and infrequent. This action results in a more conservative statement of assets but has no current effect upon cash.

It will be noticed that the financial statements are in a different form than in previous years. The change was made to conform with recently adopted reporting guidelines for colleges and universities. The major change is to distinguish in the statement between unrestricted revenues and their use and revenues which have been designated or "restricted" for specific programs and their use. The results for 1974 have been restated to be in form comparable to those for 1975.

The continuation of loss operations of whatever magnitude is never welcome. However, to the extent such losses are being incurred to provide for more effective year-round use of the plant and personnel of the Laboratory, they can be viewed as an investment aimed at creating the conditions which should bring revenues and expenditures into balance.

Alexander T. Daignault
Treasurer

The following is a statement of the auditors:

To the Trustees of Marine Biological Laboratory, Woods Hole, Massachusetts:

We have examined the balance sheet of Marine Biological Laboratory as of December 31, 1975 and the related statements of changes in fund balances and current funds revenues, expenditures, and other changes for the year then ended. Our examination was made in accordance with generally accepted auditing standards and, accordingly, included such tests of the accounting records and such other auditing procedures as we considered necessary in the circumstances. We previously examined and reported upon the financial statements of the Laboratory for the year 1974.

In our opinion, the aforementioned financial statements (with investments stated at cost) present fairly the financial position of Marine Biological Laboratory at December 31, 1975 and 1974, and its current funds revenues, expenditures and other changes for the years then ended, and the changes in its fund balances for the year ended December 31, 1975, in conformity with the accounting principles referred to in the notes to the financial statements applied on a basis consistent with that of the preceding year.

The summary of investments included herein was obtained from the Laboratory's records in the course of our examination and, in our opinion, is fairly stated in all material respects in relation to the basic financial statements taken as a whole.

Boston, Massachusetts
April 2, 1976

COOPERS & LYBRAND

MARINE BIOLOGICAL LABORATORY

Balance Sheet

December 31, 1975 and 1974

Assets	*1975*	*1974*
Current Funds:		
Unrestricted:		
Cash	$ 49,066	$ 78,195
Accounts receivable, net of allowance for uncollectible accounts of $20,000 in 1975	292,477	269,087
Inventories (Note B)		53,841
Other assets	5,927	7,668
Due to restricted current funds	(255,931)	(485,493)
Due (to) from invested funds	(26,708)	6,437
Total unrestricted	64,831	(70,265)
Restricted:		
Cash	37,909	751
Investments, at cost; market value: 1975—$1,169,931; 1974—$258,388 (Note A, Schedule I)	1,170,009	318,700
Due from unrestricted current funds	255,931	485,493
Total restricted	1,463,849	804,944
Total current funds	$ 1,528,680	$ 734,679
Invested Funds:		
Cash	23,571	2,446
Investments, at cost; market value: 1975—$3,880,277; 1974—$3,639,698 (Note A, Schedule I)	3,852,003	4,193,877
Due (to) from unrestricted current fund	26,708	(6,437)
Total invested funds	$ 3,902,282	$ 4,189,886
Plant Fund:		
Land, buildings and equipment, at cost (Note C)	12,378,202	12,454,336
Less accumulated depreciation	3,248,932	3,007,343
Total plant fund	$ 9,129,270	$ 9,446,993

The accompanying notes are an integral part of the financial statements.

MARINE BIOLOGICAL LABORATORY

Balance Sheet

December 31, 1975 and 1974

Liabilities and Fund Balances	*1975*	*1974*
Current Funds:		
Unrestricted:		
Accounts payable and accrued expenses	$ 96,396	$ 59,394
Advance subscription payments	49,037	46,690
Fund balance (deficit)	(80,602)	(176,349)
Total unrestricted	64,831	(70,265)
Restricted:		
Fund balances:		
Unexpended gifts and grants	1,387,044	735,017
Unexpended income (Note E)	76,805	69,927
Total restricted	1,463,849	804,944
Total current funds	$1,528,680	$ 734,679
Invested Funds:		
Endowment funds (Note E)	2,001,133	1,986,889
Quasi-endowment funds	1,462,894	1,804,605
Retirement fund (Note D)	438,255	398,392
Total invested funds	$3,902,282	$4,189,886
Plant Fund:		
Invested in plant	9,129,270	9,446,993
Total plant fund	$9,129,270	$9,446,993

The accompanying notes are an integral part of the financial statements.

MARINE BIOLOGICAL LABORATORY

Statement of Current Funds Revenues, Expenditures, and Other Changes

for the years ended December 31, 1975 and 1974

	Unrestricted	*Restricted*	*1975 Total*	*1974 Total*
Revenues:				
Instruction:				
Tuition	$ 128,200	$ 7,200	$ 135,400	$ 109,673
Grants and contracts:				
Government	46,316	196,233	242,549	212,708
Private		91,923	91,923	48,128
Research:				
Laboratory rentals	371,523		371,523	397,756
Grants and contracts:				
Government	47,510	163,931	211,441	275,199
Private	80,887	298,182	379,069	200,584
Dormitory	258,788		258,788	219,673
Dining hall	150,775		150,775	127,766
Library	92,851		92,851	79,516
Biological Bulletin	70,670		70,670	70,446
Support departments:				
Research services	87,712		87,712	58,393
Supply	93,488		93,488	90,914
Investment income	148,704	92,299	241,003	206,059
Gifts	50,684	102,696	153,380	83,036
Other	35,341		35,341	26,747
Total revenues	1,663,449	952,464	2,615,913	2,206,598

	Unrestricted	*Restricted*	*1975 Total*	*1974 Total*
Operating expenditures:				
Instruction	75,721	236,611	312,332	208,347
Research	47,550	464,186	511,736	516,256
Scholarships and stipends		108,590	108,590	99,859
Dormitory	112,859		112,859	93,010
Dining hall	134,053		134,053	115,873
Library	173,402	16,588	189,990	154,521
Biological Bulletin	112,143		112,143	57,474
Support departments:				
Research services	205,529		205,529	177,261
Supply	225,003	401	225,404	185,225
Administration	397,569		397,569	283,600
Plant operation	501,201	125,718	626,919	394,908
Other		370	370	
Total expenditures	1,985,030	952,464	2,937,494	2,286,334
Loss from operations	321,581		321,581	79,736
Transfers and additions:				
Excess of restricted gifts and grants received over amounts expended		733,913	733,913	575,118
From plant fund—proceeds of sale of real estate	75,000		75,000	
Utilized in current operations—(to) from restricted current fund and quasi-endowment funds	342,328	(75,008)	267,320	(59,102)
Net transfers and additions	417,328	658,905	1,076,233	516,016
Net increase in fund balances	$ 95,747	$658,905	$ 754,652	$ 436,280

The accompanying notes are an integral part of the financial statements.

MARINE BIOLOGICAL LABORATORY

STATEMENT OF CHANGES IN FUND BALANCES

for the year ended December 31, 1975

	Current Funds		*Invested Funds*			
	Unrestricted	*Restricted*	*Endowments*	*Quasi-Endowments*	*Retirement Fund*	*Plant Fund*
Revenues and other additions:						
Unrestricted current fund revenues	$1,663,449					
Grants		$ 786,363				
Gifts		968,350				
Realized net gains on sale of investments			$ 14,244			
Investment income		99,177			$ 13,659	
Proceeds of sale of real estate						$ 75,000
Addition to pension fund					44,870	
Tuition		7,200				
Total revenues and other additions	1,663,449	1,861,090	14,244		58,529	75,000
Expenditures and other reductions:						
Instruction, research and general expenditures	1,985,030	952,464				
Realized losses on sales of investments				$ 74,391		
Disposal of plant assets						36,703
Indirect costs recovered		174,713				
Payments to pensioners					18,666	
Depreciation						281,020
Total expenditures and other reductions	1,985,030	1,127,177		74,391	18,666	317,723
Transfers among funds—additions (deductions):						
Proceeds of sale of real estate	75,000					(75,000)
Utilized in current operations	342,328	(75,008)		(267,320)		
Total transfers	417,328	(75,008)		(267,320)		(75,000)
Net increase (decrease) for the year	95,747	658,905	14,244	(341,711)	39,863	(317,723)
Fund balances (deficit) at beginning of year	(176,349)	804,944	1,986,889	1,804,605	398,392	9,446,993
Fund balances (deficit) at end of year	$ (80,602)	$1,463,849	$2,001,133	$1,462,894	$438,255	$9,129,270

The accompanying notes are an integral part of the financial statements.

MARINE BIOLOGICAL LABORATORY

Notes to Financial Statements

A. *Significant Accounting Policies:*

Basis of Presentation—Fund Accounting

The 1975 financial statements have been prepared in accordance with recently adopted reporting guidelines for colleges and universities. Accordingly, the 1974 financial statements have been reclassified for comparative purposes (see also Note E).

In order to ensure observance of limitations and restrictions placed on the use of resources available to the Laboratory, the accounts of the Laboratory are maintained in accordance with the principles of "fund accounting". This is the procedure by which resources are classified into separate funds in accordance with activities or objectives specified. In the accompanying financial statements, funds that have similar characteristics have been combined.

Externally restricted funds may only be utilized in accordance with the purposes established by the source of such funds. However, the Laboratory retains full control over the utilization of unrestricted funds. Restricted gifts, grants, and other restricted resources are accounted for in the appropriate restricted funds. Restricted current funds are reported as revenue when expended for current operating purposes. Unrestricted revenue is accounted for in the unrestricted current fund.

Endowment funds are subject to restrictions requiring that the principal be invested and only the income utilized. Quasi-endowment funds have been established by the Laboratory for the same purposes as endowment funds; however, any portion of these funds may be expended.

Investments

Investments purchased by the Laboratory are carried at cost. Investments donated to the Laboratory are carried at fair market value at date received.

Investment Income and Distribution

Investment income is recorded when received and is comprised of income from the investments of specific funds and the pooled investment account. Income from the pooled investment account is distributed to the participating funds on the basis of the market value at the beginning of the quarter, adjusted for the cost of any additions or disposals during the quarter.

B. *Inventories:*

During 1975, it was decided that the Laboratory's inventory of scientific bulletins and books held for resale had no readily determinable commercial value and, accordingly, the $53,841 inventory cost was written off as a charge to current operations.

C. *Land, Buildings and Equipment:*

Following is a summary of the plant fund assets:

Classification	*1975*	*1974*
Land	$ 639,693	$ 674,693
Buildings	10,148,461	10,171,445
Equipment	1,590,048	1,608,198
	12,378,202	12,454,336
Less accumulated depreciation	3,248,932	3,007,343
	$ 9,129,270	$ 9,446,993

The original cost of land, buildings and related initial furnishing equipment is capitalized when the assets are acquired. The cost of subsequent additions and purchases, repairs and remodeling is expensed when incurred.

Depreciation is computed using the straight-line method over estimated useful lives of 40 years for buildings and 20 years for equipment.

D. *Retirement Fund:*

The Laboratory has a noncontributory pension plan for substantially all full-time employees which complies with the requirements of the Employee Retirement Income Security Act of 1974. The actuarially determined pension expenses charged to operations in 1975 and 1974 were $43,353 and $43,103, respectively. The Laboratory's policy is to fund pension costs accrued.

E. *Reclassification of Endowment Fund—Unexpended Income:*

It was determined that $65,715 of the endowment funds balance at January 1, 1974 actually represented unexpended restricted income which should have been included in current restricted funds. This amount has been treated as a correction of the current restricted fund—unexpended income balance as of January 1, 1974.

F. *Pledges and Grants:*

As of December 31, 1975, approximately $887,000 remains to be received from previous gifts and grants for specific research and instruction programs, and is expected to be received as follows:

1976	$512,000
1977	135,000
1978	135,000
1979	105,000
	$887,000

SCHEDULE I

MARINE BIOLOGICAL LABORATORY

SUMMARY OF INVESTMENTS

December 31, 1975

	COST		*MARKET*		*1975 Investment Income*
	Amount	*%*	*Amount*	*%*	
Invested Funds:					
U. S. Government securities	$ 303,237	7.9	$ 306,122	7.9	$ 6,620
Corporate bonds	1,023,682	26.6	822,485	21.2	50,768
Common stocks	1,866,650	48.4	2,122,833	54.7	94,218
Commercial paper	525,000	13.6	525,000*	13.5	46,579
Preferred stocks	115,885	3.0	86,288	2.2	4,230
Real estate	17,549	.5	17,549*	.5	
Total	$3,852,003	100.0	$3,880,277	100.0	202,415
Less custodian fees					6,714
					195,701
Current Restricted Funds:					
U. S. Government securities	1,162,321	99.3	1,162,243	99.3	
Certificate of deposit	7,688	.7	7,688*	.7	
Total	$1,170,009	100.0	$1,169,931	100.0	65,839
Total investment income					$261,540
Disposition of investment income:					
Utilized in current operations:					
Unrestricted (for general use)					148,704
Restricted (for scholarships)					92,299
Unexpended—restricted for scholarships					6,878
Retirement fund					13,659
					$261,540

* At cost.

Reference: *Biol. Bull.*, **151**: 96–107. (August, 1976)

UREASE FROM THE LUGWORM, *ARENICOLA CRISTATA*

LYNN COOLEY,[1] DANA R. CRAWFORD,[2] AND STEPHEN H. BISHOP[3]

The Marine Biological Laboratory, Woods Hole, Massachusetts 02543

Urea can arise from arginine and purine catabolism. Small amounts of urea are found in sea water and can be used by phytoplankton for growth (Mitamura and Saijo, 1975; McCarthy, 1972). In animals containing urease (urea amidohydrolase, EC 3.5.1.5), urea can serve as a source of ammonia. Although ammonia is considered an end product of nitrogen metabolism in aquatic invertebrate animals, ammonia can also play a role in ion regulation (Maetz, 1975), osmoregulation (Gilles, 1975), buffering of blood and urine (Campbell, 1973), calcification (Speeg and Campbell, 1968; Crossland and Barnes, 1974), and some developmental processes (Epel, Steinhardt, Humphreys, and Mazia, 1974).

Urease is found in some marine molluscs (Hanlon, 1975), polychaetes (Hult, 1969; Campbell, 1973; Razet and Retière, 1967), cestodes (Bishop, 1975; Simmons, 1961), and starfish (Brookbank and Whiteley, 1954). The gastric urease activity seen in vertebrates is generally associated with the microflora and is not considered to be of animal origin (Delluva, Markley and Davis, 1968; Rahman and Decker, 1966). In the medicinal leech, the low level of kidney urease activity is associated with *Corynebacterium* sp. living in the lumen fluid (Büssing, Döll and Freytag, 1953). Because the properties of bacterial ureases are varied, demonstration that the urease activity found in an animal's tissue is of animal origin is often difficult. In studies with the land snail (Speeg and Campbell, 1968), the urease was shown to be unique in that its properties differed from those of the urease of commensal microorganisms.

Except for the studies with the land snail urease (McDonald, 1970) no attempt at purification or characterization of animal ureases has been reported. Additionally, in describing the ureases from invertebrate sources, assays are rarely standardized so that a distinction between animal and microbial ureases can be made. This report describes the partial purification and characterization of lugworm (*A. cristata*) gut tissue urease and is the first step in determining the organismic origin of this important ammonia-forming enzyme.

METHODS AND MATERIALS

Lugworms, *Arenicola cristata* Stimpson, were provided by the Supply Department of the Marine Biological Laboratory, Woods Hole, Massachusetts or were purchased from NEMSCO, Bourne, Massachusetts. All lugworms were collected in the area south of Cape Cod and were held in running seawater tables for several days prior to use.

---

[1] Present address: Connecticut College, New London, Connecticut.

[2] Present address: University of Massachusetts, Harbor Campus College II, Dorchester, Massachusetts.

[3] Present address: Department of Biochemistry, Baylor College of Medicine, Houston, Texas 77030 and to whom correspondence should be addressed.

TABLE I

Partial purification of urease from A. cristata *gut.*

Preparation	Volume (ml)	A_{280}	Units	Total activity	Specific activity*
Dialyzed extract	62	10.6	2.4	148	0.23
DEAE-cellulose column (+)	10	11.3	10.4	104	0.92
Sephadex G-150 (+)	11	0.5	6.8	75	13.60

* Specific activity is units/A_{280}.
(+) Combined fractions.

Nessler's reagent, DEAE-cellulose, reducted glutathione and Tris (hydroxymethyl) aminomethane were purchased from Sigma Chemical Company. Reagents for scintillation counting were purchased from New England Nuclear Company. Sephadex G-50, G-150, aldolase, ovalbumin, and ribonuclease were purchased from Pharmacea Fine Chemicals. Iodoacetamide, acetohydroxamic acid, N-ethylmaleimide, and hydroxyurea were purchased from ICN Pharmaceuticals, Incorporated and prepared in ice water just before used. [^{14}C] urea with a specific radioactivity of 55 mCi/mmole was purchased from Schwarz-Mann. The [^{14}C] urea was diluted with [^{12}C] urea to a specific radioactivity of approximately 0.5 μCi/μmole. All other reagents were reagent grade from Fischer Scientific Company or Baker Chemical Company. All pH measurements were made at 22° C with a Beckman Zeromatic pH meter. Unless otherwise stated, all reagent solutions were prepared in distilled-deionized water.

During purification, the enzyme was assayed using the previously described procedure (Bishop, 1975) at pH 7 in 0.1 M potassium phosphate with 20 mM urea at 30° C. Ammonia formation was determined by Nesslerization after diffusion. A radiometric assay (McDonald, Speeg, and Campbell, 1972) was used for all kinetic and inhibitor studies. For the radiometric assay, the enzyme was incubated with the [^{14}C] urea solutions in Erlenmeyer flasks (25 ml) closed with gum rubber stoppers equipped with polyethylene cups containing 0.2 ml of 1 M hyaminehydroxide in methanol. Reactions were terminated by addition of 1 ml of N H_2SO_4 and the evolved CO_2 trapped in the hyaminehydroxide. Ten μmoles of $NaHCO_3$ were added to the reaction mixture as carrier for the [^{14}C]CO_2 in all the radiometric assay incubations. After shaking for 1 hr at room temperature, the cup was transferred to a scintillation counting vial containing 15 ml of toluene which contained 5 g of 2,5-diphenyloxazole and 0.2 g of 1,4-bis [2-(5-phenyloxazolyl)] benzene per liter. Radioactivity was determined using a Beckman LS 230 liquid scintillation spectrometer. Correction for self absorption was made using the external standard method.

Protein was estimated by determining A_{280} using a Guilford Spectrophotometer. A unit of enzyme activity was the amount of enzyme required for conversion of urea to a μmole of ammonia/hr under the conditions of assay as described above. Specific activity was units/A_{280} of enzyme.

The Sephadex G-150 and DEAE-cellulose was prepared and the columns packed as described previously (Bishop, Barnes, and Kirkpatrick, 1972). The Sephadex G-150 column was calibrated for the estimation of molecular weights

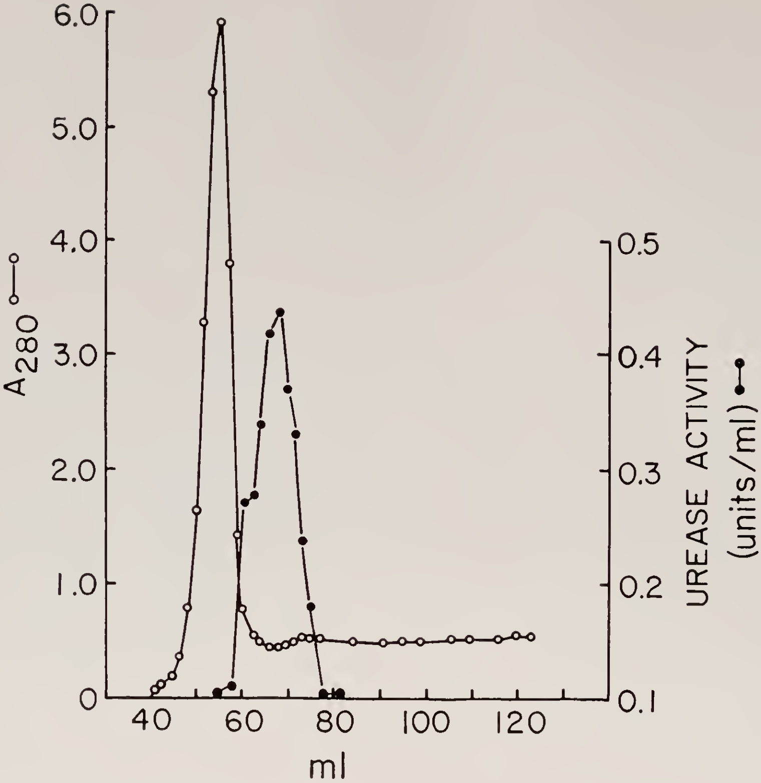


FIGURE 1. Chromatography of urease from *A. cristata* gut tissue on Sephadex G-150. Conditions are described in text. Horizontal axis describes ml of elutant and vertical axis describes absorbancy at 280 nM of the elutant. A_{280} and urease activity are indicated by the open and closed circles, respectively.

as described by Andrews (1964). Polyacrylamide gel electrophoresis was performed as described by Davis (1964) using Tris-glycine buffer at pH 8.3. No stacking gels were used and the gels were pre-electrophoresed to remove persulfate. Electrophoresis of the enzyme preparation was performed at 4° C. Staining was with Coomasse Blue.

RESULTS

During preparation of the enzyme, all procedures were performed at 0–4° C. Centrifugations were for 15 min at 15,000 rpm using an SS-34 rotor in a Sorvall RC-2B refrigerated centrifuge. Gut tissue behind the pharynx was removed from specimens of *A. cristata* and the tissue was washed thoroughly with running sea water. The tissue was homogenized in 9 ml of 0.05 M Tris-hydrochloride (pH 7.3)-1 mM reduced glutathione per gram of tissue in a ground glass homogenizer. After centrifugation the supernatant fluid was decanted and dialyzed twice for 4–8 hrs against ten volumes of 5 mM Tris-hydrochloride (pH 7.9). After dialysis, the preparation was passed through a DEAE-cellulose column previously equilibrated with the same buffer. In a typical preparation, about 5 grams of tissue were used and the DEAE-cellulose column had a 2 × 5 cm packed wet bed volume. After addition of the enzyme, the DEAE-cellulose column was washed with 100 ml of buffer from the second dialysis. The column was then washed with 0.05 M Tris-hydrochloride (pH 7.3)-0.2 M KCl. The urease activity eluted with the solvent front and was collected in test tubes. Tubes with urease were combined and the protein concentrated by addition of 5.5 g $(NH_4)_2SO_4$/10 ml of solution. The precipitated protein was collected by centrifugation and dissolved in 2 ml of 0.05 M Tris-hydrochloride (pH 7.3)–0.2 M KCl. This preparation was applied to a Sephadex G-150 column (2 × 50 cm), previously equilibrated with the same buffer, and the column was washed at a flow rate of 12 ml/hr. The enzyme activity eluted in a symetrical peak just behind a large A_{280} peak at the void volume (Fig. 1.). The elution profile from the Sephadex column was calibrated by applying 1 ml of a buffer solution containing 4 mg each of adlolase (160,000 MW), ovalbumin (45,000 MW) and ribonuclease (13,700 MW). The apparent molecular weight of the *A. cristata* gut urease was 200,000. When Sephadex G-150 chromatography was repeated using sodium phosphate (50 mM; pH 7)-sodium acetate (100 mM), the same molecular weight was obtained. The purification procedure resulted in an enrichment of 70 fold in specific activity with a 50–60% recovery of the original urease activity (Table I).

Approximately 40 μg of partially purified urease was mixed in 25% glycerol in 0.02M Tris-glycine (pH 8.3) containing a trace of Bromphenol Blue. This mixture was applied to a 6% polyacrylamide gel column (0.7 × 8 cm) and the proteins separated by electrophoresis using 150 volts and 12 milliamps/tube. After tracking dye reached the anodal end of the gel column, the gel was removed and sliced

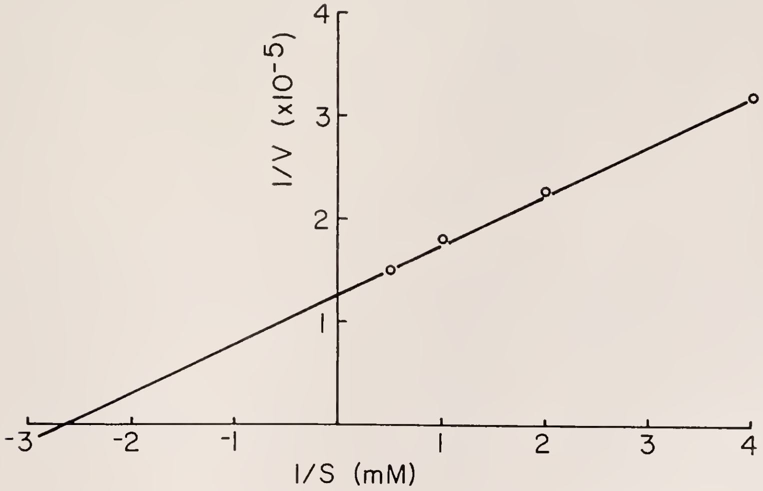


FIGURE 2. Variation in the velocity of *A. cristata* gut urease reaction with urea concentration. The reaction mixture contained the indicated amount of [^{14}C] urea (0.5 μCi/angle) 100 μmoles of potassium phosphate (pH 7.1), and enzyme in 1 ml using the radiometric assay. All incubations were at 30° C for 20 min.

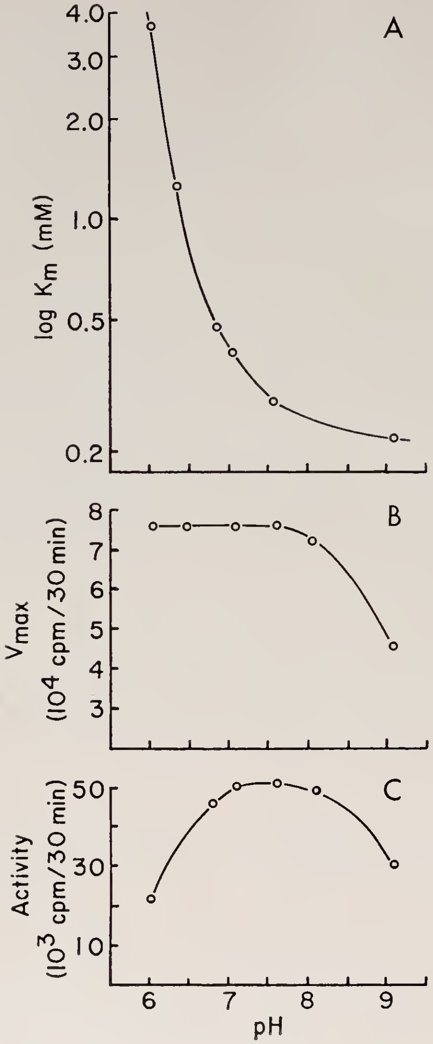


FIGURE 3. Variation in the activity of urease from *A. cristata* gut tissue with change in pH. Horizontal axis describes the pH and the vertical axis describes the activity or K_m depending upon the figure. The buffer contained 0.2 M Tris and 0.2 M KH_2PO_4 mixed in equal portions and adjusted to the appropriate pH with HCl or KOH. Each point in Figure 3A and 3B was determined from the intercepts of double reciprocal plots (Fig. 2) at the indicated pH using the radiometric assay. The reaction mixture in Figures 3A and 3B contained 100 μmoles of the Tris-phosphate buffer at the appropriate pH, between 0.2 and 5 μmoles of [^{14}C] urea (0.5 μCi/μmole), and enzyme in 1 ml. In Figure 3C, the reaction mixture contained 2 μmoles of [^{14}C] urea (0.5 μCi/μmole), 100 μmoles of Tris-phosphate buffer at the appropriate pH and enzyme in 1 ml. All incubations were at 30° C.

longitudinally. One longitudinal piece was stained with Coomassee Blue. The other piece was sliced in 0.5 cm cross sections and each section assayed for urease activity using the radiometric assay. After destaining, at least four weakly staining blue bands and three major bands were evident. Urease activity was associated with a major band at R_f 0.35.

The partially purified preparation which had been tested for purity by polyacrylamide gel electrophoresis was used for the kinetic and inhibitor studies reported below. The enzyme could be frozen and thawed or freeze-dried and dissolved without loss of activity. The partially purified enzyme preparation was routinely stored at −20° C. Enzyme preparations held in solution in phosphate buffer (pH 7) for 2 weeks lost 40–60% of their activity. All activity was lost if the enzyme was boiled for 2 min. In the crude state, no activity was lost when held at 52° C for 3 min. When tissue homogenates prepared in Tris buffer containing 0.8 M sucrose or 0.5 M KCl were centrifuged, all urease activity was detected in the supernatant fluid fraction. The tissue activity of the enzyme averaged within 10% of 30 units/g wet weight in five preparations.

In preliminary studies, [^{14}C] CO_2 liberation from [^{14}C] urea increased linearly with increased incubation time to 40 min and in proportion to the amount of enzyme added to the reaction mixture. The pH for maximal activity was between pH 6.5 and pH 8. Using the radiometric assay, the urease activity showed saturation kinetics with respect to the urea concentration. The apparent K_m for urea was 0.38 mM in potassium phosphate buffer at pH 7.1 (Fig. 2). The K_m's for urea at pH 7.5 in 0.1 M Tris-acetate, potassium phosphate, or Tris-phosphate were all

TABLE II

Inhibition of urease from A. cristata *gut tissue. The reaction mixture contained the indicated amount of inhibitor, 100 µmoles Tris-acetate pH 7.5, 25 µg of protein, and 2 µmoles of [^{14}C] urea (0.5 µCi/µmole). Enzyme was prepared by passage of 5 mg of the partially purified preparation through a Sephadex G-50 column (40 × 2 cm) equilibrated with Tris acetate (0.02 M) pH 7.5 to remove glutathione and phosphate. In the preincubated samples, the mixture was incubated for the indicated time period without the [^{14}C] urea and the reaction started by addition of the [^{14}C] urea. In the preparations with no preincubation, the reaction was started by addition of the enzyme. The percent inhibition was determined by difference from complete reaction mixtures after a 30 min incubation using the preparations containing no added inhibitors. All incubations were at 30° C.*

Inhibitor added	Concentration mM	Per cent inhibition		
		With preincubation	(min)	No preincubation
None		0	(15)	0
Iodoacetamide	1.0	0	(15)	0
$AgNO_3$	1.0	100	(15)	100
	0.1	97	(15)	97
N-ethylmaleimide	1.0	89	(15)	67
	0.1	42	(15)	22
Hydroxylamine	1.0	93	(15)	33
	0.1	22	(15)	7
Hydroxyurea	1.0	97	(15)	32
	0.1	26	(15)	0
	0.1	49	(30)	0
Acetohydroxamate	5.0	100	(30)	96
	1.0	98	(30)	77
	0.1	98	(30)	22

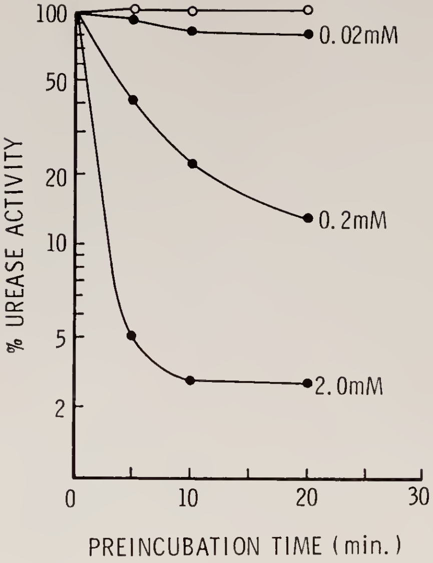


FIGURE 4. Inactivation of urease from *A. cristata* gut tissues by acetohydroxamate. The reaction mixture contained the indicated amount of acetohydroxamate (closed circles), 100 μmoles of potassium phosphate (pH 7.3), and enzyme in a 1 ml volume. Control flasks (open circles) contained no inhibitor. After incubation for the indicated time period, remaining urease activity was determined by addition of 2 μmoles of [^{14}C] urea (0.5 μCi/mole). The enzymatic reaction was terminated after a 30 min incubation and the evolved [^{14}C]CO_2 determined as described in the text. All incubations were at 30° C.

within 10% of 0.3 mM. The variation in K_m for urea-enzyme complex was determined at pH values between 6 and 9.1 (Fig. 3). The apparent K_m decreased markedly between pH 6 and pH 7 and was lowest (0.38–0.22 mM) between pH 7 and pH 9 (Fig. 3A). The V_{max} was constant between pH 6 and pH 7.6 but decreased between pH 8 and pH 9.1 (Fig. 3B). The reaction velocities measured at various pH values and at an arbitrary substrate concentration of 2 mM (Fig. 3C) were maximal between pH 7 and pH 8 in the Tris-phosphate buffer system.

Because HCl was used to adjust the pH of the buffers between pH 7.6 and pH 6 and because of the dramatic change in K_m(urea) in this pH range, it was essential to determine whether Cl^- was a competitive inhibitor. Assays were performed between 0.25 and 2 mM urea at 135 mM NaCl or twice the maximal concentration achieved in the pH experiment (Fig. 3) in Tris-phosphate buffer (pH 7.5). Although there was some noncompetitive inhibition (10% at V_{max}), there was no change in K_m for urea.

The inhibition by some sulfhydryl reactive reagents and compounds which inhibit urease activity was evaluated to further discriminate between *A. cristata* gut urease and urease from other sources (Table II). These agents behaved as irreversible inhibitors. To determine whether they were active site directed, inhibition or inactivation studies were undertaken with enzyme incubated in the presence and absence of the substrate (Table II). Iodoacetamide did not inhibit and no protection from Ag^+ inactivation was offered by incubation with the substrate. By contrast, substrate protection was afforded against N-ethylmaleimide inactivation. Hydroxylamine, hydroxyurea, and acetohydroxamate all inhibited the enzyme and substrate protection was observed. Acetohydroxamate was a more effective inhibitor than either hydroxyurea or hydroxylamine.

The sensitivity of the enzyme to acetohydroxamate inactivation was evaluated by incubating the enzyme with various concentrations of acetohydroxamate for various time periods. Inactivation proceeded in a nonlinear fashion (Fig. 4) with time and approached a maximum degree of inhibition which was dependent upon inhibitor concentration.

Discussion

A. cristata gut urease is a reasonably stable enzyme, exists as a single soluble entity and has unique properties which distinguish it from microbial, plant, and other animal ureases.

With jack bean urease, the pH optimum is between pH 6 and pH 8 (Blakeley, Hinds, Kunze, Webb and Zerner, 1969; Lynn, 1967; Sundaram and Laidler, 1970) and the K_m for urea is 4–6 mM between pH 5 and 7 and 2–2.5 mM between pH 8.0 and 9. The bacterial ureases appear to have more varied properties but are less well studied. With *Bacillus pasteurii* (Larson and Kallio, 1954), the K_m falls from 100 mM and 130 mM at pH 5.7 and pH 6.7 to 40 mM at pH 7.7. Urease from rumen microorganisms showed a pH optimum between pH 8 and 9 and a K_m of 1.5 mM at pH 8.5 (Rahman and Decker, 1966). In *Corynebacterium renale*, the urease had a pH optimum between pH 7–8 and a K_m of 30 mM at pH 7 (Lister, 1956). In *Proteus mirabilis*, the urease activity was greatest between pH 6 and 8.3 and had a K_m of 10 mM at pH 7 (Anderson, Kopko, Diedler, and Nohle, 1969). Essentially the same properties have been reported for ureases from other *Proteus* species (Hase and Kobashi, 1967; Magna-Plaza, Montes, and Ruiz-Herrera, 1971; Speeg and Campbell, 1968). With urease from *Aerobacter aerogenes*, the K_m for urea increases from 1.5 to 6 mM as the pH decreases from 8 to 6.5 in phosphate buffer (Kamel and Hamed, 1975). Kinetic properties of ureases from animal sources have been examined in only three instances. Ureases in cestode species (Bishop, 1975; Simmons, 1961) have K_m's between 5 mM and 15 mM at the optimal pH of 7–7.5 in phosphate or tris-maleate buffer. The snail urease, on the other hand, has a low K_m of 0.1 mM at the optimal pH of pH 8.5, (McDonald, 1970; McDonald and Campbell, 1970). The variation of K_m with pH has not been evaluated for the cestode or snail enzymes.

In general, then, the microbial and plant ureases have K_m's for urea in the 1.5–250 mM range with the lower K_m's at the higher pH's. *A. cristata* gut urease exhibits K_m's in the optimal pH 7–9 range which are one-fifth to one-eighth of the lowest reported K_m's for urease from any microbial or plant source. The K_m is very similar to that reported for the snail enzyme (McDonald and Campbell, 1970). The sharp decrease in K_m between pH 6 and pH 7 suggests that a functional group on the enzyme with a pK between pH 6 and pH 7 is involved in substrate binding (Fig. 3). The broad pH optimum with a decline in V_{max} between pH 8 and pH 9 suggests that a functional group with a pK in this range is involved in the catalytic mechanism.

With regard to mechanism, we could find no evidence for the ATP-biotin dependent urease found in green algae and some fungi (Roon and Levenberg, 1968; Thompson and Muenster, 1971).

The inhibition by hydroxylamine, hydroxyurea and acetohydroxamate observed for the *A. cristata* gut urease (Table II) is similar to the inhibition observed with all other ureases (Blakeley, Hinds, Kunze, Webb, and Zerner, 1969; Fishbein and Carbone, 1965; Fishbein, Winter, and Davidson, 1965; Gale, 1965, 1966; Gale and Atkins, 1969; Hase and Kobashi, 1967; Kobashi, Takebe, Terashima, and Hase, 1975; McDonald and Campbell, 1970; Speeg and Campbell, 1968). From the data in Figure 4 an I_{50} of about 5×10^{-5} M can be calculated for the *A. cristata* gut urease. This I_{50} is similar to that found with the jack bean and *Proteus* urease (Blakeley, Hinds, Kunze, Webb and Zerner, 1969; Fishbein and Carbone, 1965; Hase and Kobashi, 1967) but somewhat greater than the I_{50} found for the snail enzyme (McDonald, 1970). The *A. cristata* gut urease is similar to the snail enzyme in its reactivity of sulfhydryl reactive agents (McDonald, 1970). Neither are inhibited by iodoacetamide but both are strongly inhibited by Ag^+ and N-ethylmaleimide (Table II). The jack bean urease is also strongly inhibited by N-ethylmaleimide and Ag^+ (Gorin and Chin, 1965).

The molecular weight by gel filtration is somewhat lower than the 262,000 daltons reported for the snail enzyme (McDonald and Campbell, 1970) and substantially lower than the 482,000 daltons of the α-form of jack bean urease (Blakeley, Webb, and Zerner, 1969; Fishbein, 1975). Walberg (1957) reports a molecular weight of 473,000 for *Proteus* urease. Gel-filtration experiments indicate that this high molecular weight form of urease is probably predominant in other bacterial species (Kamel and Hamed, 1975; Magana-Plaza *et al.*, 1971). However, Tanis and Naylor (1968) have reported low molecular weight forms of urease in the 230,000 MW range from *Proteus* and plant sources including jack beans. Fishbein (1969) has confirmed the existance of the 240,000 MW form of jack bean urease as one of the isozyme forms of this enzyme, but he considers the parent form to be the 482,000 MW form. Jack bean urease can form aggregates or dissociate according to ionic strength, pH, glycol concentration, and thiol concentration (Fishbein, 1975). With *A. cristata* gut urease, no evidence for isozyme forms was obtained from the gel-filtration and electrophoretic experiments.

The characteristic properties of the *A. cristata* gut urease—low K_m for urea, large variation of K_m with pH, pH optimum, molecular weight, and sensitivity to inhibitors—distinguish this urease from the urease found in cestodes, the land snail, plants and microorganisms. From this preliminary characterization study, the *A. cristata* gut urease would appear to be a unique animal urease.

Not all invertebrate animals have urease activity in their gut or other tissues and the presence of urease seems unrelated to habitat or phylogeny. Present views (Campbell, 1973) seem to favor some relationship between urease function and ammonia production. As mentioned in the introduction, ammonia can play a role in pH adjustment, ion regulation, and osmoregulation in addition to nitrogen excretion. If the urease is of animal origin, then its synthesis should be under metabolic control and may be related to the regulation of ammonia formation. For instance, in the land crab, *Cardisoma guanhumi*, the uric acid which accumulates in the hepatopancreas while the animal is on land, is systematically degraded to ammonia, carbon dioxide and glyoxylate when the animal enters the water (Gifford, 1968).

The induction or regulation of the purinolytic mechanism and urease has not been investigated in marine invertebrates. In the lugworm, the presence of urease, arginase (Bishop and Crawford, unpublished results) and the purinolytic pathway (Razet and Retière, 1967) means that the arginine and purine derivatives in the tissues can serve as sources of ammonia for ion regulation or for amino acid biosynthesis in the adjustment of the intracellular osmotic pressure during salinity changes in the seawater environment.

Part of this research effort was performed during the Experimental Invertebrate Zoology Course at the Marine Biological Laboratory (1975). It was supported in part by the Laboratory and grants from the NSF (BMS-74-10433), NIH (HL-17372) and The Robert A. Welch Foundation (Q-294). Thanks are expressed to Dr. M. J. Greenberg (Course Director) for encouragement and assistance in preparation of the manuscript. A preliminary report has appeared (Cooley, Crawford, and Bishop, 1975). Contribution No. 57 from the Tallahassee, Sopchoppy and Gulf Coast Marine Biological Association.

SUMMARY

Urease from animal tissues is often considered to be of microbial rather than animal origin. A determination of key properties of urease isolated from an animal tissue should permit an assessment of the origin of the enzyme. Lugworm (*A. cristata*) gut urease was purified seventy fold from tissue homogenates by chromatography on DEAE-cellulose and Sephadex G-150. The apparent molecular weight by gel-filtration was 200,000. The K_m for urea declined from about 3.5 mM at pH 6 to 0.38 mM at pH 7 then decreased with increasing pH to 0.2 mM at pH 9 in Tris-phosphate buffer. The V_{max} was constant between pH 6 and 8 then declined above pH 8. N-ethylmaleimide, $AgNO_3$ but not iodoacetamide inhibited enzyme activity. Acetohydroxamate, hydroxyurea, and hydroxylamine inhibited in the manner similar to the inhibition seen with ureases from other sources. The characteristic properties of *A. cristata* gut urease—low K_m, pattern of variation of K_m with pH, molecular weight, and sensitivity to inhibitors—distinguish this urease from urease in bacteria, plants, land snails and cestodes.

LITERATURE CITED

ANDERSON, J. A., F. KOPKO, A. J. DIEDLER, AND E. G. NOHLE, 1969. Partial purification and properties of urease from *Proteus mirabilis*. *Fed. Proc.*, **28** : 764.

ANDREWS, P., 1964. Estimaton of molecular weights of proteins by Sephadex gel-filtration. *Biochem.* J., **91** : 222–223.

BISHOP, S. H., 1975. Ammonia formation and amino acid excretion by *Gyrocotyle fimbriata* (Cestoidea). *J. Parasitol.*, **61** : 79–88.

BISHOP, S. H., L. B. BARNES, AND D. S. KIRKPATRICK, 1972. Adenosine deaminase from *Metridium senile* (L.), a sea anemone. *Comp. Biochem. Physiol.*, **43B** : 949–963.

BLAKELEY, R. L., E. C. WEBB, AND B. ZERNER, 1969. Jack bean urease (EC 3.5.1.5). A new purification and reliable rate assay. *Biochemistry*, **8** : 1984–1990.

BLAKELEY, R. L., J. A. HINDS, H. E. KUNZE, E. C. WEBB, AND B. ZERNER, 1969. Jack bean urease (EC 3.5.1.5). Demonstration of a carbamoyl-transfer reaction and inhibition by hydroxamic acids. *Biochemistry*, **8** : 1991–2000.

BROOKBANK, J. W., AND A. H. WHITELEY, 1954. Studies of the urease of the eggs and embryos of the sea urchin, *Strongylocentrotus purpuratus*. *Biol. Bull.*, **107** : 57–63.

Büsing, K. H., W. Döll, and K. Freytag, 1953. Die Bakterienflora der medizineschen Blutegel. *Arch. Mikrobiol.*, **19**: 52–86.

Campbell, J. W., 1973. Nitrogen excretion. Pages 279–316 in C. L. Prosser, Ed., *Comparative animal physiology*, 3rd Ed., Saunders, Philadelphia.

Cooley, L., D. R. Crawford, and S. H. Bishop, 1975. Urease from the lug-worm, *Arenicola cristata*. *Biol. Bull.*, **149**: 424–425.

Crossland, C. J., and D. J. Barnes, 1974. The role of metabolic nitrogen in coral calcification. *Mar. Biol.*, **28**: 325–332.

Davis, B. J., 1964. Disc-electrophoresis II. Method and application to human serum proteins. *Ann. N. Y. Acad. Sci.*, **121**: 404–427.

Delluva, A. M., K. Markley, and R. E. Davis, 1968. The absence of gastric urease in germ-free animals. *Biochim. Biophys. Acta*, **151**: 646–650.

Epel, D., R. Steinhardt, T. Humphreys, and D. Mazia, 1974. An analysis of the partial metabolic depression of sea urchin eggs by ammonia: the existence of independent pathways in the program of activation at fertilization. *Develop. Biol.*, **40**: 245–255.

Fishbein, W. N., 1969. The structural basis for the catalytic complexity of urease: interacting and interconvertible molecular species (with a note on isozyme classes). *Ann. N. Y. Acad. Sci.*, **147**: 857–881.

Fishbein, W. N., 1975. Structural classes of jack bean urease variants and their relation to a structural classification of isozymes. Pages 403–417 in C. Market, Ed., *Isozymes, I*, Academic Press, Inc., New York.

Fishbein, W. N., and P. P. Carbone, 1965. Urease catalysis. II. Inhibition of the enzyme of hydroxyurea, hydroxylamine and acetohydroxamic acid. *J. Biol. Chem.*, **240**: 2407–2414.

Fishbein, W. N., T. S. Winter, and J. D. Davidson, 1965. Urease catalysis. I. Stoichiometry, specificity and kinetics of a second substrate: Hydroxyurea. *J. Biol. Chem.*, **240**: 2402–2406.

Gale, G. R., 1965. Inhibition of urease by hydroxyurea. *Biochem. Pharmacol.*, **14**: 693–698.

Gale, G. R., 1966. Urease activity and antibiotic sensitivity. *J. Bacteriol.*, **91**: 499–506.

Gale, G. R., and L. M. Atkins, 1969. Inhibition of urease by hydroxamic acids. *Arch. Int Pharmacodyn. Ther.*, **180**: 289–298.

Gifford, C. A., 1968. Accumulation of uric acid in the land crab, *Cardiosoma guanhumi*. *Amer. Zoologist*, **8**: 521–528.

Gilles, R., 1975. Mechanisms of ion and osmoregulation. Pages 259–347 in O. Kinne, Ed., *Marine Ecology* Vol. II, J. Wiley and Sons, New York.

Gorin, G., and C. C. Chin, 1965. Urease. IV. Its reaction with N-ethylmaleimide and with silver ion. *Biochim. Biophys. Acta*, **99**: 418–426.

Hanlon, D. P. 1975. The distribution of arginase and urease in marine invertebrates. *Comp Biochem. Physiol.*, **52**: 261–264.

Hase, J., and K. Kobashi, 1967. Inhibition of *Proteus vulgaris* urease by hydroxamic acids. *J. Biochem.*, **62**: 293–299.

Hult, J. E., 1969. Nitrogenous waste products and excretory enzymes in the marine polychaete *Cirriformia spirabranchia* (Moore, 1904). *Comp. Biochem. Physiol.*, **31**: 15–24.

Kamel, M. Y., and R. R. Hamed, 1975. *Aerobacter aerogenes* PRL-R3 urease. Purification and properties. *Acta Biol. Med. Germ.*, **34**: 971–979.

Kobashi, K., S. Takebe, N. Terashima, and J. Hase, 1975. Inhibition of urease activity by hydroxamic acid derivatives of amino acids. *J. Biochem.*, **77**: 837–843.

Larson, A. D., and R. E. Kallio, 1954. Purification and properties of bacterial urease. *J. Bacteriol.*, **68**: 67–73.

Lister, A. J., 1956. The kinetics of urease activity in *Corynebacterium renale*. *J. Gen Microbiol.*, **14**: 478–484.

Lynn, K. R., 1967. Some properties and purification of urease. *Biochim. Biophys. Acta*, **146**: 205–218.

Maetz, J., 1975. Aspects of adaptation to hypo-osmotic and hyperosmotic environments. Pages 1–167 in D. C. Malins and J. R. Sargent, Eds., *Biochemical and biophysical perspectives in marine biology*, Vol. 1, Academic Press, Inc., New York.

Magaña-Plaza, I., C. Montes, and J. Ruiz-Herrera, 1971. Purification and biochemical characterization of urease from *Proteus rettgeri*. *Biochemi. Biophys. Acta*, **242**: 230–237.

McCarthy, J. J., 1972. The uptake of urea by natural populations of marine phytoplankton. *Limnol. Oceanogr.*, **17**: 738–748.

McDonald, J. A., 1970. Invertebrate urease: purification and characterization of urease from a land snail. *Ph. D. Thesis, Rice University*, Houston, Texas. (Diss. Abstr., **31B**: 3160B; Order no. 70–23,553.)

McDonald, J. A., and J. W. Campbell, 1970. Invertebrate urease: Purification and properties of urease from a land snail. *Fed. Proc.*, **29**: 904.

McDonald, J. A., K. V. Speeg, and J. W. Campbell, 1972. Urease: A sensitive and specific radiometric assay. *Enzymologia*, **42**: 7–9.

Mitamura, O., and Y. Saijo, 1975. Decomposition of urea associated with photosynthesis of phytoplankton in coastal waters. *Mar. Biol.*, **30**: 67–72.

Rahman, S. A., and P. Decker, 1966. Comparative study of the urease in the rumen wall and rumen content. *Nature*, **209**: 618–619.

Razet, P., and C. Retière, 1967. Recherche des enzymes de la chaine de l'uricolyse chez les annelides, polychetes. *C. R. Hebd. Séanc. Acad. Sci. Paris Ser. D.*, **264**: 356–359.

Roon, R. J., and B. Levenberg, 1968. Adenosine triphosphate-dependent avidin-sensitive enzymatic cleavage or urea in yeast and green algae. *J. Biol. Chem.*, **243**: 5213–5215.

Simmons, J. E., Jr., 1961. Urease activity in trypanorhynch cestodes. *Biol. Bull.*, **121**: 535–546.

Speeg, K. V., and J. W. Campbell, 1968. Formation and volatilization of ammonia gas by terrestrial snails. *Amer. J. Physiol.*, **214**: 1392–1402.

Sundaram, P. V., and K. J. Laidler, 1970. The urease-catalyzed hydrolysis of some substituted ureas and esters of carbamic acid. *Can. J. Biochem.*, **48**: 1132–1140.

Tanis, R. J., and A. W. Naylor, 1968. Physical and chemical studies of a low-molecular weight form of urease. *Biochem. J.*, **108**: 771–777.

Thompson, J. F., and A. M. E. Muenster, 1971. Separation of the *Chlorella* ATP: urea amido-lyase into two components. *Biochem. Biophys. Res. Commun.*, **43**: 1049–1055.

Walberg, C. B., 1957. The purification and properties of bacterial urease. *Ph. D. Dissertation, University of Southern California School of Medicine*, Los Angeles, California.

Reference: *Biol. Bull.*, **151**: 108–125. (August, 1976)

SEASONAL GROWTH AND REPRODUCTION OF AN INTERTIDAL SPONGE, *HALICLONA PERMOLLIS* (BOWERBANK)

DAVID W. ELVIN[1]

Marine Science Center, School of Oceanography, Oregon State University, Newport, Oregon 97365

Relatively little information is available on the ecological factors which influence the sequence of events leading to sexual reproduction in sponges (Fell, 1974a). The importance of temperature in regulating the seasonal breeding of marine invertebrates has long been suggested, and the subject is reviewed by Kinne (1970). Reproductive studies on sponges have related the presence of gametes, embryos, and larvae to annual temperature changes in the environment. In a field of study of *Haliclona ecbasis* from San Francisco Bay, Fell (1970) found gametes and embryos present from August to November when sea water temperatures were above 12° C. Hartman (1958), using larval settling as an index of sexual reproduction in a Long Island Sound population of *Haliclona loosanoffi*, found settling to occur when the temperature reached 20° to 22° C.

A few quantitative studies have been made on the seasonal changes in the partitioning of nutritive resources into growth and reproductive potential. Reiswig (1973) studied growth and reproduction in a Jamaican population of *Mycale* sp., and Stone (1970) followed changes in the rock surface covered by the encrusting sponge, *Hymeniacidon perleve*, simultaneously noting the proportion of the population containing embryos.

With the exception of Stone's work, the above studies were carried out on populations which were continuously submerged rather than intertidal populations which undergo fluctuating conditions. Furthermore, if one is to elucidate the mechanisms controlling reproduction in the field, precise analysis of both environmental regimes and reproductive processes is necessary. This paper presents methods for quantifying gamete production of an encrusting intertidal sponge and for estimating the true tissue temperature during the period of tidal exposure. Reproductive ouput and growth rates are then described for a population of *Haliclona permollis* located on the Central Oregon Coast. The biological observations are discussed in relation to the nutritive, salinity, light and thermal regimes of the environment.

Materials and Methods

Sampling procedure

The sponge, *Haliclona permollis*, was chosen because it is a cosmopolitan species (de Laubenfels, 1936) available all year in the easily accessible intertidal region and forms flat incrustations which are conducive to measurement. Furthermore, since it is a sessile, filter-feeding animal, its physical and nutritive environment can be estimated at all times. Ten to 22 pieces of sponge were collected monthly (bimonthly during spring) at stations from the rocky intertidal (+1 to +2 feet above MLLW) at Yaquina Head, Oregon over a four year period. By marking the collection site with a metal stake and recording the location of the sponges, it was possible to sample the same mass of sponge in several cases for as long as four months. However, most individuals had disappeared or grew together with their neighbor after one to two months and thus lost their identity. The collected specimens were immediately placed in Bouin's fixative. Later they were embedded in paraffin, sectioned vertically at 10 μ thickness, and stained with haematoxylin and eosin.

---

[1] Present address: Department of Zoology, Marsh Life Science Building, University of Vermont, Burlington, Vermont 05401.

Quantification of gametes, embryos, and larvae

Fifteen 1.9 mm^2 microscopic sections of each sample were observed and the number of eggs, embryos, larvae, and sperm packets were counted and averaged for that month. An estimate of the number of reproductive entities per mm^3 in the above categories was obtained for each specimen by modification of a formula used by Abercrombie (1941) to compare nuclear populations of different tissues. The formula is Number per $mm^3 = N\ (t/D + t)\ (53)$, where N is the average number of gametes or embryos observed in the microscopic field, t is the thickness of the section (10 μ), and D is the diameter of the component counted. The constant, 53, is a factor converting the volume of observation (0.019 mm^3) to one mm^3. Only those eggs with a nucleus showing were counted. The average number of gametes or embryos per mm^3 in the population was calculated on the basis of both the total number of specimens of a particular sex and the total population.

Calculation of oocyte and embryo production rates

In order to achieve a dynamic description of reproduction, it is necessary to calculate the rates of conversion into the stages leading to larval production. The methods employed in studies on cell renewal systems by Olive (1971) were applied to sponges. For any time interval the change in the number of cells in the oocyte (ΔN_O), embryo (ΔN_E), and larval (ΔN_L) compartments can be described by the differences in the rates associated with each compartment: $\Delta N_O = R_O - R_E - R_{D1}$; $\Delta N_E = R_E - R_L - R_{D2}$; and $\Delta N_L = R_L - R_S - R_{D3}$. The rates are in units of number of eggs (R_O), embryos (R_E), or larvae (R_L) produced and the number of larvae released (R_S) per day per mm^3 of sponge. Data indicated that for every four oocytes produced, only one reaches the embryonic stage. Thus the rate of oocyte resorption (R_{D1}) approximates $3R_E$. Since the maximum density of embryos approximates that of the larvae, and since there is no sign of distintegrating larvae, the remaining rates of resorption (R_{D2} and R_{D3}) can be considered negligible. The rate of larval formation is much slower than that of egg formation. Thus the various rates can be computed using the difference between the monthly samples by evaluating the following formulas: $R_O = \Delta N_O + 4\Delta N_E$; $R_E = \Delta N_E + \Delta N_L$; and $R_L = \Delta N_L + R_S$. A relative value for the annual production of eggs (P_O) was calculated in units of number per cm^2 per year for an average sponge 2.5 mm in thickness by the formula $P_O = R_O$ (total reproductive days) (% females) (100) (2.5). A similar formula was used for establishing total embryos produced using R_E instead of R_O.

Estimates of somatic growth

Somatic growth was studied in terms of both the increase in biomass and the increase in substrate covered by a sponge. In the first case, the presence of active growth areas as defined using the criteria of Simpson (1968) was noted. For this mesenchymal index the specimens were scored values from 0 to 3 corresponding to a few loosely packed mesenchymal amoebocytes (0), low numbers of mesenchymal amoebocytes (1), low density mesenchyme with tracts or clumps of amoebocytes (2), and high density of mesenchymal amoebocytes (3).

Two dimensional growth by spreading over the substrate was estimated in the field by measuring the increase in the area of the flat encrusting sponges over a two week period. The outline of the sponge was traced on a piece of transparent plastic ten times and transferred to heavy paper. The shapes were cut out, weighed, and averaged to get an estimate of area. Growth was determined as the difference in areas. Since growth is related to the mass of available tissue and since the sponges studied had a fairly uniform thickness (2 to 3 mm), increases in area were divided by the total area to obtain a specific growth rate. Animals obviously broken or eaten as well as those which had grown together with neighboring individuals were eliminated from consideration.

Laboratory observations on the effects of temperature on gamete production

Sponges attached to rocks were collected in February, 1972, before any signs of gametogenesis were apparent and placed in 30 liter tanks of U.V. filtered sea water held at 4°, 7°, 9.5°, and 13° C. The tanks received approximately eight hours of fluorescent light a day. The food source for the experimental sponges was the flagellate, *Isochrysis galbana*, maintained at a concentration of about 10^5 cells/liter. Three monthly samples of the sponges were taken and processed for histological observation. On the 77th day the temperature in the 13° C tank was lowered to 10° C.

Collection of environmental data

Water and air temperatures were continuously recorded by thermoprobes installed at Whale Cove, Oregon, during the years 1970 and 1971 (Gonor and Thum, 1970). Other temperature data was obtained from the U. S. Weather Bureau at Yaquina Bay and from Yaquina Head at the time of specimen collection. Relative humidity was calculated from the difference in readings of wet and dry bulb thermometers on the days of collection. Time of exposure of the +1 foot level was obtained from water level recordings of the U. S. Weather Bureau station. Actual tissue temperatures of the sponges were measured to within 0.1° C with a hypodermic thermoprobe (Yellow Springs Instrument Co.). Total short wave insolation up to 4 μ was measured with an unshielded horizontal Eppley pyroheliometer. Salinity values for the surface sea water were obtained from Gonor, Thum, and Elvin (1970). Values for the amount of rain falling on the exposed sponges were calculated from data of the U. S. Weather Bureau.

The amount of chemically oxidizable particulates per liter of sea water was used as a measure of available nutrients. Sea water was collected up to four times each month, filtered through a 200 μ mesh screen, and collected on a 0.2 μ mesh

Teflon filter. The samples were oxidized using the dichromate method of Strickland and Parsons (1968) with glucose as a standard. To a second liter of sea water, 5 ml of Lugol's iodine solution was added, and the sample was allowed to stand a few days at 4° C. The settled diatoms were then identified and counted.

Calculation of tissue temperature

Tissue temperatures of sponges exposed by low tide were estimated by fitting the observed temperatures into an equation describing the animal at thermal equilibrium, $K_1 (T_{sp} - T_{air}) + K_2 (aT_{sp}^2 + bT_{sp} + c)(1 - Rh) = K_3 (L)$, where T_{sp} is the tissue temperature of the sponge, and $K_1 (T_{sp} - T_{air})$ is a combined term for conduction and longwave back radiation (Hutchinson, 1957). The term $K_2 (aT_{sp}^2 + bT_{sp} + c)(1 - Rh)$ is an expression for heat loss by evaporation incorporating a term for vapor pressure as a function of temperature in mm Hg multiplied by a term for relative humidity. $K_3 (L)$ is a term for heating by solar radiation. The constants K_1, K_2, and K_3 were determined by fitting measured sponge temperatures and concurrent environmental conditions into the equation. The evaporative characteristics of the sponge were determined under several conditions in the laboratory using a chamber in which temperature and humidity could be regulated. This formula for tissue temperature during exposure is an empirical one fitting the observed data, and not all the potential parameters of a heat budget were considered. Thus during exposure, the tissue temperature of a sponge for any relative humidity (Rh) is given by the quadratic solution to the heat budget equation $T_{sp} = -\frac{1}{2} Z + \sqrt{\frac{1}{4} Z^2 + ZT_{air} + 12 Z (L) - 240}$, where Z is a combination of the constants equal to $162.2/(1 - Rh)$, and L is insolation in langleys per minute. A regression of 10 pairs of predicted and observed data gave $T_{predicted} = 0.3 + 0.94 T_{observed}$, and at the 95% confidence level predicted temperatures were within 0.64° C of observed values.

A continuous temperature was then calculated for a hypothetical sponge population every hour of the day for one year. When a sponge was submerged, its temperature was that of sea water; and when exposed, the above formula was used employing a value of 80% for the relative humidity. From the continuous temperature data three thermal parameters were chosen as possibly having correlations with physiological events leading to larval production. Average daily temperature, daily maximum and minimum temperatures, and time spent above a threshold temperature were calculated for 15 day intervals.

RESULTS

Histological samples

Examination of the histological samples enables one to determine the time of gametogenesis, the sex ratio, the rates of gamete production, and the total number of gametes produced. Single oocytes are distributed throughout the mesenchyme while sperm packets are found in clumps of three or four. Embryos are generally located near the base of the sponge often in clusters. During those periods when oocytes and embryos were most abundant the standard error of their mean densities as determined by counting 15 sections of a single specimen were on the order of

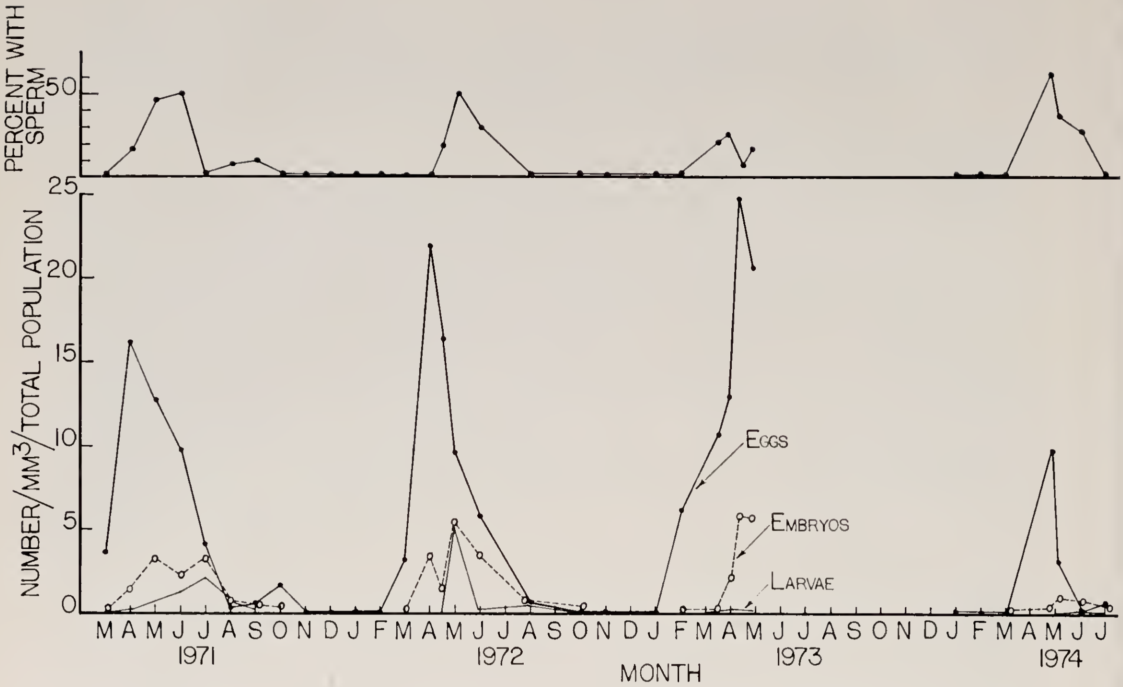


FIGURE 1. Percentage of specimens in the monthly samples containing sperm masses (upper), and the average density of eggs, embryos, and larvae observed for the total sponge population (lower). Data was not collected during the period, May to December, 1973.

5 oocytes/mm^3 and 2 embryos/mm^3. Within a population sample of 10 specimens the standard errors were 4 oocytes/mm^3 and 2 embryos/mm^3 for means near 20 oocytes/mm^3 and 5 embryos/mm^3. The rather high variation in embyro densities reflects a clumping tendency within the sponge.

Figure 1 shows the monthly occurrence of eggs, embryos, and larvae in the total sponge population and the percentage of specimens with sperm masses. Oocytes were first seen in the March samples during 1971 and 1972. However, in 1973 they were present as early as February 13, and in 1974 they did not appear until April 26. The maximum oocyte density for 1974 is also lower than the previous three years;

TABLE I

Sex ratio in late spring populations of Haliclona permollis *at Yaquina Head, Oregon.*

Year	Collection dates	N	Males	Females	Indifferent	Chi²	Significance*
1971	May–June	25	12	13	0	0	No
1972	May–June	13	5	7	1	0.16	No
1973	April	24	3	20	1	9.6	Yes
1974	April–May	32	15	9	8	1.04	No

* Significant difference from a ratio of 1:1 at the 5% level.

TABLE II

Rates of oocyte and embryo production per female Haliclona permollis *at Yaquina Head, Oregon.*

Year	Period	Rates (number/mm³/day)	
		Oocytes	Embryo
1971	Mar–April	+1.30	+0.07
	April–May	+0.49	+0.14
1972	Feb–Mar	+0.30	0
	Mar–April	+1.90	+0.23
	April–April	+0.77	−0.03
	April–May	+0.11	+0.52
1973	Jan–Feb	+0.32	0
	Feb–Mar	+0.31	0
	Mar–Mar	+0.53	+0.39
	Mar–April	+1.43	+0.28
	April–April	−0.24	0
1974	Mar–April	+0.71	+0.01
	April–May	−2.00	+0.22

however, this decrease reflects a change in the sex ratio rather than a change in the number of oocytes produced per female. During the first three years the earliest embryos were seen in April approximately one month after the appearance of oocytes. Larvae are first observed in May, but their pattern of release differs over the years. Some sperm masses were usually present by the time embryos were produced; however, the maximum sperm density was generally found in May, one month following maximum embryo density. In 1972 a high embryo density was found in the April sample which did not contain males.

The number of males and females in the monthly samples at a time when the sex was known for a majority of the specimens is presented in Table I. In 1971 and 1972 the sex ratio was 1:1, but in 1973 there were significantly more females than males. In contrast there were either fewer females or an abnormally large number of sponges which did not produce any gametes in 1974. Over the entire four years only one (0.7%) simultaneous hermaphrodite was found among the 147 specimens exhibiting gametes out of a total of 342 specimens. In 1974, 17 specimens were collected in which the microhabitat could be definitely classified as totally shaded or totally exposed to the sun during low tide. While 37% of the males were on rocks exposed to the sun during low tide, only 11% of the females were found in such a situation.

Calculated rates of egg and embryo production are given in Table II. The maximal rates appear to be between 1 and 2 eggs/mm^3/day/female and 0.2 to 0.5 embryos/mm^3/day/female. Since the variabilities of the counts upon which the rates are based are high, the order of magnitude is of greater significance than the actual values. In Table III, we see that while the total number of eggs in females is nearly a constant 44/mm^3, over the years the number of embryos varies eightfold. The difference in relative annual production of embryos for the whole population rises to fifteen times when fluctuations in the sex ratio are considered.

TABLE III

Annual production of oocytes and embryos for the Haliclona permollis *at Yaquina Head, Oregon.*

Year	Female production (number/mm³/year)		Percentage of females	Relative annual production in total population (number/cm²/year)	
	Oocytes	Embryos		Oocytes	Embryos
1971	44	5.7	52	5730	745
1972	46	19.6	54	6190	2640
1973	47	8.4	83	9750	1780
1974	38	2.4	28	280	177

Constant temperature experiments

Table IV presents the results of the laboratory observations on the reproductive state of specimens after 77 days at four constant temperatures. Clearly 4° C for any extended period is a lethal condition. The number of specimens which did produce gametes was too small to show significant differences between the various temperatures but some information can be gained from the results. It is clear that sperm can be formed at any temperature between 7° and 13° C, although sperm packet density was much greater at 13° C. Spermatogenesis always began after oogenesis. Oocytes were produced at the rates of 0.04 and 0.16 oocytes/mm³/day for temperatures of 7° and 9.5° C, respectively, but these rates are only a tenth of those in the field. Embryos were never produced although males and females were in the same tanks. Most interesting was the appearance of abnormal oocytes in the sample in which the temperature was reduced from 13° to 9.5° C. These oocytes differed from normal eggs by having, in addition to the nucleolus, a large eosinic granule in the nucleus. Sponges which did not produce gametes were found to be in a state of regression.

Observations on growth

The observations on the somatic growth indicators are presented in Figure 2. Mesenchymal densities with an index above 1.2 were considered to be significantly

TABLE IV

Histological characteristics of sponges after 77 days submergence at constant temperatures in the laboratory.

(February 14 to May 2, 1972)

Temperature (° C)	N	Mesenchymal index	Number of oocytes	Date of first appearance	Number of sperm	Date of first appearance	Number without gametes
4	4	1.4*	0	—	0	—	4
7	6	1.0	2	Mar 25	2	May 2	2
9.5	8	1.7	2	Mar 2	2	May 2	4
13	6	0.7	0**	—	4	May 2	2**

* Died within 30 days.
** Abnormal oocytes and two hermaphrodites produced *after* cooling to 10° C.

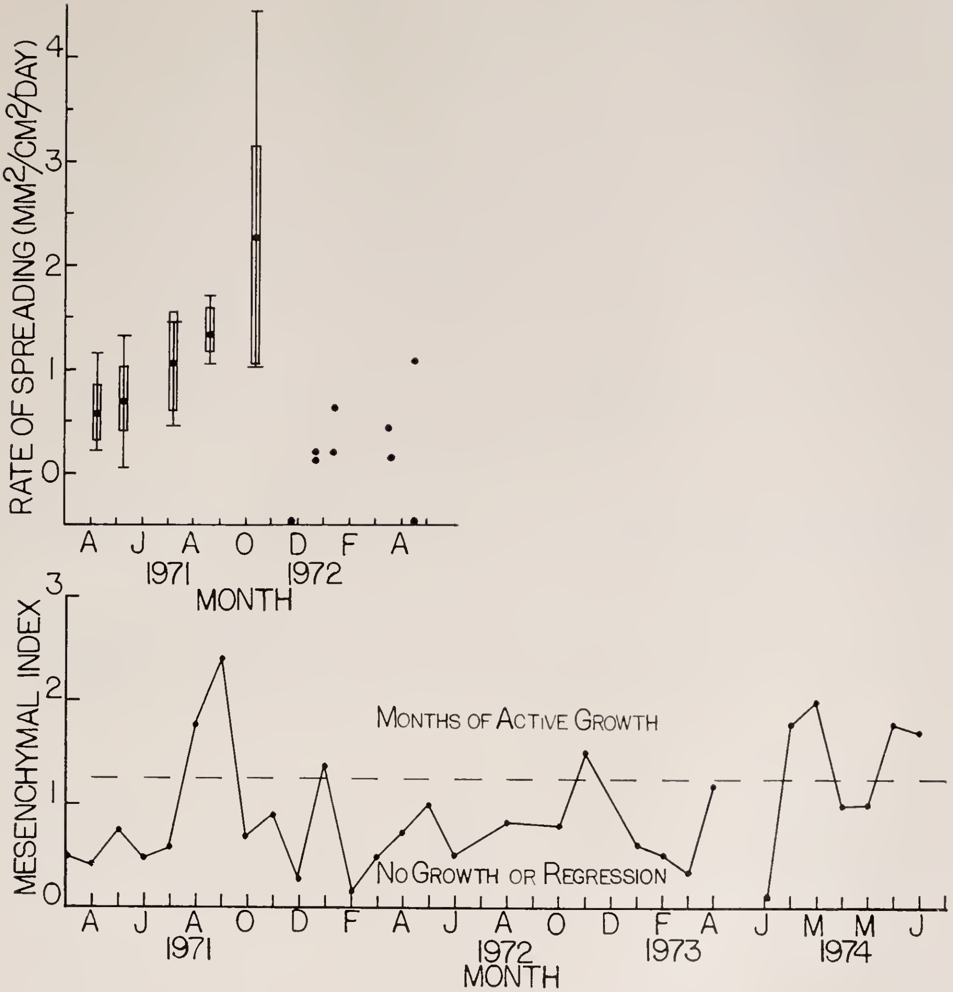


FIGURE 2. Rate of spreading or increase in area covered by a sponge (upper) showing average, 90% confidence intervals, and ranges. Dots indicate values for single individuals. The average mesenchymal index (lower) for monthly samples is shown in which specimens with loosely packed cells were given a value of 0, with low density amoebocytes a value of 1, with clumps and tracts of amoebocytes a value of 2, and high density amoebocytes a value of 3. Values above the dashed line (index = 1.2) demonstrate a significant increase in mesenchymal density.

different from nongrowth values. In 1971, increase in the mesenchymal amoebocytes in August and September preceded spreading by one month. During late fall of 1972 there was again an increase in mesenchymal density. 1974 appears to be an abnormal year since an increase in amoebocytes occurred during February. An average spreading rate during summer and fall is on the order of 1 $mm^2/cm^2/day$. During November spreading suddenly ceased and in some cases regression was measured. From October to December the rate of specimen disappearance from the

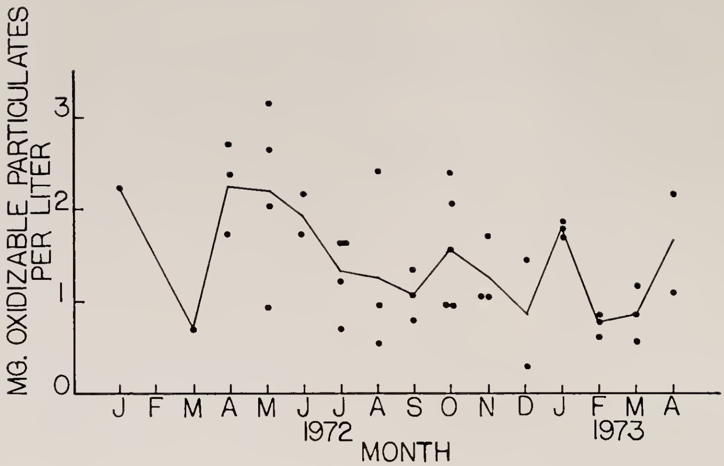


FIGURE 3. Chemically oxidizable particulate material (0.2 to 200 μ) suspended in the near-shore water in units of mg glucose per liter. Monthly averages are connected by a line and the values of samples collected one week apart are represented by dots.

marked field specimens was about 1.5% per day, while in April and May it dropped to 0.86% per day. Production of gemmules was not observed in histological sections although gemmules from this species were occasionally present in the field.

Environmental factors

Growth and reproductive trends were studied in relation to the annual salinity, nutrient, light, and tissue temperature regimes. Extensive rain, especially during tidal exposure, results in hypo-osmotic conditions. The potential rainfall on exposed sponges was calculated by multiplying the total inches per day by the fraction of the day during which exposure takes place. The heaviest rainfall occurs from November through February. During January and February surface seawater salinity may drop as low as 20‰ on particular days, but the rest of the year the values stay between 30 and 34‰. The crucial period of February to April had significantly lower rainfall (13 inches) in 1973 compared to 1972 (26.7 inches) and 1974 (29.1 inches). Long periods of exposure in the sun during May to July when the low tides occur during the middle of the day lead to dessication and consequently hyper-osmotic conditions.

Nutrients available to the sponge population as measured by the amount of oxidizable particulate material ranging in size from 0.2 to 200 μ had values between 0.2 and 3.2 mg glucose equivalents per liter (Fig. 3). High values in the spring are associated with diatom blooms increasing from 10^3 cells per liter to 10^4 and 10^5 cells per liter beginning April 1, 1972 and April 11, 1973, respectively. Occasionally in the fall and winter terrestrial runoff and wave mixing of bottom detritus result in high amounts of both oxidizable and inorganic particulates. It is clear from Figure 3 that monthly averages have little significance in light of the possible threefold difference between two successive weeks.

The characteristics of sunlight falling on the +1 foot level during tidal exposure are a function of both the tidal regime and incident solar insolation. For this collecting site the value of impinging sunlight rises rapidly in March and reaches a maximum in May.

Intertidal sponges have three possible thermal regimes. The submerged sponge has a tissue temperature always equal to that of sea water. The sponge subjected during tidal exposure to direct sunlight is consequently heated, and the sponge in the shade during tidal exposure undergoes slow heating or cooling depending on air temperature, wind speed, and humidity. An example of a rapid change in tissue temperature is shown in Figure 4 in which a sponge was initially exposed in the shade for several hours and then exposed to the sun shortly before it was covered by the incoming tide. In this case, heating was a rapid 13° C per hour. A nearby specimen in the shade remained at the wet bulb temperature.

Continuous tissue temperatures of hypothetical sponges were calculated for the year 1970 to 1971. For the continuously submerged population, 24 hourly temperatures each day were combined to give an average daily temperature, and these daily temperatures were averaged for 15 day intervals. The result is an average temperature remaining within two degrees of 10° C (Fig. 5). Sponges exposed in the shade or sun have average values within 1° C of the submerged values. These deviations from the submerged average are small due to the relatively short 0 to 6 hour periods of exposure each day at the +1 foot level, and there

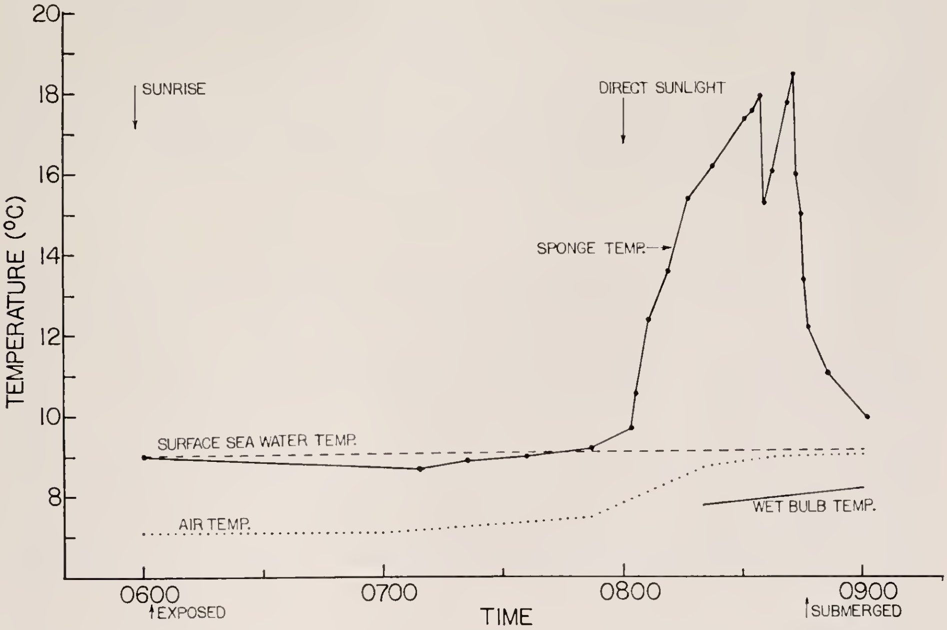


FIGURE 4. Tissue temperature of a sponge which has been exposed by the outgoing tide. Initial exposure was in the shade and direct exposure to sunlight occurred at 0800 hours. The sudden decrease at 0830 hours was caused by a large wave. A nearby sponge in the shade remained at the wet bulb temperature.

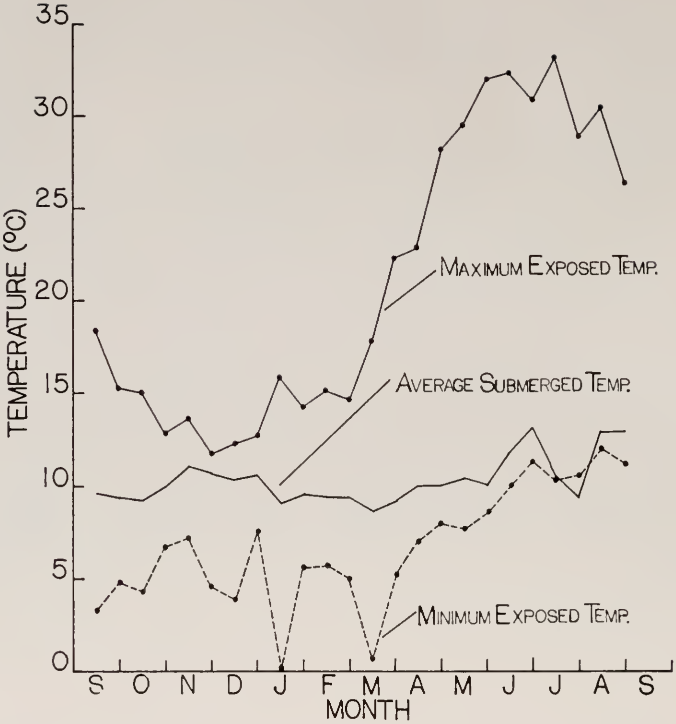


FIGURE 5. Annual tissue temperatures of sponges showing the average temperature of submerged populations and the maximum and minimum temperatures reached each 15 day period by exposed populations. Average tissue temperatures of the exposed populations were found to be within a degree of the submerged group and are not shown. This data covers the period 1970 to 1971.

is no significant difference between the average temperatures of the three regimes.

As has been shown, the temperatures reached during the period of exposure can be far from those of sea water. The maximum and minimum tissue temperatures attained in each bimonthly period are also recorded in Figure 5. Heating during tidal exposure occurs in late March, and temperature differences of 10° C begin to occur at this time. The minimum temperatures approaching 0° C in January and March should not be neglected in an analysis of the thermal regime. A better indication of the thermal regime can be obtained by calculating the amount of time spent above arbitrary threshold temperatures. Threshold values of 10° and 12° C demonstrated significant annual changes in late March and in late May, respectively, for those sponges exposed directly to sunlight. Although maximum exposed temperatures of 32° C were predicted for sponges exposed during the summer, very little time was spent above 14° C. The great variation during late summer and fall reflects intermittent periods of cooler water due to coastal upwelling. Average values for sea water and air temperatures during the years 1971 to 1974 were compared, and it was found that both air and sea water temperatures during February of 1973 were 1.5° to 2.0° C warmer than those of other years.

Relationship between environmental events and reproductive behavior

The relationship between environment and reproductive events must be presented for later discussion. Oogenesis in the *H. permollis* population was first observed in late February to early March. During this period in 1971, freshwater stress from rain declined and tissue temperature was at its lowest. Particulate nutrient concentration is also at a minimum. In fact, in 1972 and 1973, eggs were formed well before the diatom bloom, and no direct relationship exists. Examination of the continuous tissue temperature calculation (Fig. 5) and consideration of the monthly values of sunlight impinging on the +1 foot level for the year 1970–1971 shows that initiation of oogenesis occurs with increase in sunlight during the February to March period rather than temperature. For the four year period, 1971 to 1974, however, comparison of the average February air and seawater temperatures with the reproductive data supports a correlation of either tissue temperature or light with the onset of oogenesis. 1973 was a year of early oogenesis and had higher temperatures, less rain, and probably increased incident radiation. During 1974, a year of late oogenesis, there was much rain and consequently reduced insolation and low temperatures.

Although the maximum rate of oocyte formation occurs in late March to early April when there is an increase in the time the sponge spends above 10° C, there is no correlation between rate of oogenesis and average tissue temperature over the years.

Spermatogenesis is better related to thermal changes than oogenesis. Sperm masses are first seen in April when both impinging light and the length of time spent above the 10° C threshold by sponges exposed to the sun increases further. Sperm were observed earlier (March 17) in the warmer year of 1973. In the laboratory, sperm were first observed on May 2, slightly later than in the field specimens. The density of sperm masses produced was greater at 13° C than at the lower temperatures, although it should be recalled a few sperm packets were produced at 7° C. There is no apparent relationship between the initiation of spermatogenesis or sperm release and the concentration of oxidizable particulates. Rapid disappearance of sperm in males occurs from late May to early July when maximum exposed temperatures exist but the sea water temperatures may be quite cool due to upwelling.

There is a positive relationship between embryo production and the amount of oxidizable particulates during the years 1971 and 1972. Embryo production, unlike oocyte development, involves accumulation of materials, and available nutrients would be expected to influence the process. Comparison of Table II and Figure 3 shows that the rates of embryo production, 0.52 and 0.28 embryos/mm^3/day, for the years 1972 and 1973, can be correlated with the average amount of oxidizable particulates in the water, 2.2 and 1.5 mg glucose/liter, respectively. The total amounts of embryos produced during these years, 19.6 and 8.4 embryos/mm^3/female, respectively, also show this relationship.

Discussion

Analysis of growth and reproduction of the intertidal sponge, *Haliclona permollis*, over a four year period reveals variation in the onset of gametogenesis and the quantities of gametes and embryos produced. The temporal and quantitative aspects of reproduction and growth can be related to changes in the impinging sunlight, nutrition, and tissue temperature of the organism. These changes in microhabitat are in turn influenced by several primary environmental factors. Both quality and quantity of particulate food are affected by terrestrial runoff, wave mixing, and spawning or death of other organisms as well as the response of primary producers to sunlight. The tissue temperature of the sponge is determined during tidal exposure by sunlight, tidal regime, relative humidity, wind speed, and the air temperature, whereas during submergence it is determined only by seawater temperatures. The existence of subpopulations with respect to the environment further complicates any interpretation of reproductive behavior.

An explanation of sexual reproduction in sponges must describe those intrinsic and environmental factors which initiate gametogenesis, control sexual differentiation, and influence the rates of gamete production. In sponges with a dominant gemmule stage, there may exist a regulatory connection between reproduction and gemmule germination (Fell, 1974b). Gilbert (1974) found oocyte formation occurred throughout the year within a week after placement of the gemmules of the freshwater sponge, *Spongilla lacustris*, into a lake and concluded that egg production was under endogenous control not requiring environmental stimuli. Fell (1974a) found for the marine species, *Haliclona loosanoffi*, that sexual reproduction was initiated very soon after gemmule germination, but he points out that this species is the only marine sponge known in which gemmules persist exclusively for part of the cycle. In contrast, on the Oregon coast, specimens of *H. permollis* with adult tissues are always present during midwinter although gemmules are occasionally found. If spring reproductive behavior were a consequence of gemmulation the previous fall, the larger number of specimens lacking sexual characteristics in 1974 might be ascribed to low gemmule production the previous year. However, such a system would not explain the reproductive success of earlier years when there was no particular increase noted in gemmules. Initiation of gametogenesis in *H. permollis* appears environmentally controlled, but the stimulus could still involve a short period of somatic growth in those sponges surviving the previous winter.

Initiation of gametogenesis is really the onset of differentiation of archaeocytes or choanocytes into primary oocytes and spermatocytes (Fell, 1974a). This differentiation and the beginning of meiosis in *H. permollis* is not a continuous process but occurs once a year for a brief period of two weeks or less in duration. Incident light during a crucial late February to early March period is the environmental parameter most closely related to the initiation of oogenesis. The correlation with increasing temperature is poor since in 1971 the period of oogenesis occurs during March, a time with the lowest average tissue temperature (9° C), but the possibility of a cold stimulus cannot be overlooked. While the less precise environmental data of 1972, 1973, and 1974 support a thermal effect, they also support the hypothesis of a light cue. Assuming that rain is an indication of cloud cover, increasing amounts of light in 1974, 1972, and 1973 correspond to earlier appearance of gametes on April 26, March 14, and February 13, respectively. The quantity of light falling on the shaded and nonshaded populations during tidal exposure will of course be different, but the trends for both populations will be the same. The increase in light level for the sponges to a maximum in May results from tidal exposure shifting to the middle of the day coupled with the insolation increasing as the year progresses. The suggestion in this paper that light directly affects sponge physiology is supported by the observations of Rasmont (1970), who found a positive effect of light on respiration and an inhibitory effect of light on the gemmulation of a freshwater sponge. Since the sponge did not possess symbiotic algae, Rasmont suggests that sponge cells themselves are photosensitive.

Many sponges can potentially express the characteristics of either sex. *Haliclona* and the related genus *Reniera* contain examples of both successive and simultaneous hermaphrodites (Fell, 1974a). The basis of this hermaphrodism could be at the genetic level or due to the coalescence of larvae as reported for *Ophlitaspongia seriata* by Fry (1970). However, only 0.7% of the *H. permollis* specimens were found to exhibit both embryos and sperm compared to the 6% found by Fell (1970) for *H. ecbasis*. The question arises as to how one sex becomes favored over the other. A previous study on *Microciona prolifera* has shown higher thermal thresholds for sperm formation than for oogenesis (Simpson, 1968). Although several independent pieces of data in this report suggest that warmer temperatures favor sperm formation in *H. permollis* and are above the optimum for oogenesis, none of them are statistically conclusive. More males and higher sperm densities occurred at 13° C than at 7° and 9.5° C, males are often seen later in the year than females when tissue temperatures are higher, and only 11% of the females in 1974 were found in positions exposed to the sun while 37% of the males were found in this microhabitat. However, there is conflicting evidence for the thermal initiation of gametogenesis. Both types of gametes were in fact formed at 7° C, and abnormal eggs were formed in males following a temperature drop. Furthermore, a comparison of the sex ratios between the years contradicts the hypothesis of thermal sex determination since more females were present during the warmer 1973 and significantly more males in the cooler 1974. Finally, the field data in this study suggest that neither rates of oogenesis nor the total amounts of eggs produced are influenced by temperature.

If sponges are hermaphroditic, and if sexual differentiation is truly thermally controlled, then a dilemma arises since in the field sponges destined to become males must pass through a period of low temperatures favorable to oogenesis before reaching that optimal temperature for sperm production. The dilemma could be solved if the population is asynchronous with respect to the initiation of gametogenesis either physiologically or due to microhabitat differences. Those sponges that begin gametogenesis early in the year form oocytes and those that begin later form sperm. Gametes once formed could inhibit further gametogenesis as was suggested for the freshwater sponges (Gilbert, 1974).

Such a system may be related to the greater bioenergetic costs of oocyte and embryo production relative to sperm production. As Figures 1 and 2 show, somatic growth and reproduction appear to be mutually exclusive. Although the rate of growth was not separated by sex, 1973, a year with many females, showed a low mesenchymal index, and 1974, a year with many males, showed a higher mesenchymal index. Clearly a larger biomass is beneficial for egg production. Those individuals which have increased in size before the reproductive period may be better suited for egg production and are induced to do so by either increasing light or temperature.

Any individual which has survived the winter and is in at least its second year would therefore have a greater chance of producing eggs. The sponges from the larvae of the previous year would be smaller and grow in the spring before producing sperm. The large number of females in 1973 could have given rise to a large surviving larval population which then became the increased number of males the following year. Such an explanation would incorporate the features of the low density and size advantage models for hermaphroditism as discussed by Ghiselin (1969). However, the situation is not that simple since as in 1974 it is possible for a large percentage of the population to have no sexual expression. In order to fully understand the sexual sequence of this animal the same individual must be sampled for at least two years. Although this study followed specimens from the same rocks for four years, it was not possible to keep track of individuals. It is clear that average tissue temperatures of these Oregon sponge populations do not have the large annual differences reported in similar studies and as a result temperature changes may not be the best cue for reproduction. In summary, the thesis that temperature is the primary controlling factor for sexual reproduction in marine sponges should be reexamined.

The appearance of embryos obviously requires the earlier presence of oocytes, but it is unclear whether embryogenesis always requires fertilization. Gilbert (1974) states that fertilization is necessary for cleavage in *Spongilla lacustris*. Neither fertilization nor cleavage stages were observed in *H. permollis* although multiple nuclei were occasionally seen in a few embryos. As Fell (1969) has shown for *H. ecbasis*, the oocytes engulf numerous nurse cells which obscure most of the detail of the oocyte activity and presumed fertilization. In 1971, 1973, and 1974 embryos were found at the time of the first appearance of sperm. The apparent presence of embryos before the appearance of sperm in 1972 may be due to the fact that these early structures are really oocytes which have engulfed a large number of nurse cells. If decrease in sperm density is any indication of the time of sperm release, then fertilization occurs from May to June.

No correlation was found between the rate of embryo production and the average air and seawater temperatures, but a positive relationship between rate of embryogenesis and the amount of oxidizable particulates in the water does exist. Embryo growth is determined by nutrients through the engulfment of nurse cells and possibly by the rate of synthesis of certain storage products. Reiswig (1972) analyzed incurrent and excurrent water from Jamaican sponges and suggests that the unresolvable particulate fraction of which colloidal materials form a major part are an important nutritive source for sponges. Therefore, the concentrations of the larger particles followed in this study may not have direct relationship to sponge nutritive uptake. During periods of high primary production or spawning by other animals, a large amount of colloidal material is also produced. The failure to produce embryos in the female laboratory specimens could have resulted either from lack of sperm release or the flagellates being an inadequate diet.

An approximation of the material allocated to reproduction can be made based on volumes. Reiswig (1973) calculated for the Jamaican sponge, *Mycale* sp., that 2.3% of the volume was involved in production of the large 2.6 mm embryos. For *H. permollis*, the smaller 0.18 mm embryos compose 1.5% of the total volume, and up to 6.3% is found in sperm masses in contrast to the 5% to 10% found by

Reiswig. The similarity between the values is remarkable in light of the different species and environments of the two populations and may reflect a physiological characteristic of the material and energy budgets of sponges. It should be noted that the amount of cellular material present in a cubic millimeter of sponge is variable. The somatic biomass certainly influences the number of embryos produced since it is related to the amount of collected and accumulated nutrients, and it should be a consideration during further quantitative study of sponge reproduction.

As would be expected, somatic growth also shows variation throughout the year. During the fall of 1971, an increase in somatic growth detected as spreading of the sponge on the rock surface occurred. Minimal growth and often regression occurred from December until April. Although maximum spreading rate was found in the fall, growth and reproductive processes overlap since some spreading did occur during the spring. The rates presented (1 $mm^2/cm^2/day$) or 1% per day are similar to the 0.3 to 0.5% found by Stone (1970) for another encrusting sponge when his area index data is converted to similar units. For the more massive *Mycale* sp. in Jamaican waters, Reiswig found an annual growth rate of 60% for mature specimens. In contrast, *H. permollis* could have three times this rate if it was allowed 100 growing days a year. Such a difference probably reflects the requirement of the temperate intertidal sponges for a fast growth rate to compensate for physical destruction during harsh winters and for grazing of limpets and nudibranches (Elvin, 1976). It will be recalled that the death rate during the winter was estimated at 1.5% per day.

During 1971 and 1972 some increase in cell density was in evidence in the spring, but growth mainly occurred in the late fall (October to November). Increases in mesenchymal index for February, 1974, a year in which oogenesis was particularly low, are interesting since rainfall that month was about four times that of the high reproductive, low growth period of 1973. In addition to hypo-osmotic stress, this stormy period also would have had high concentrations of inorganic particulates which may cause clogging of the sponge. Thus instead of a period of increased growth, it is possible that February, 1974 was a period of stress and that the rise in mesenchymal density was a result of regression rather than proliferation. During early March new individuals are generally found even though reproduction has not occurred. It cannot be stated whether these sponges result from outgrowths of remaining adult tissues in cracks of the rocks, colonies arising from larvae of the previous fall, or germination of gemmules.

Dissection of the intertidal environment of *H. permollis* over a four year period has revealed a complex set of parameters which can be related to growth, timing of reproduction, and the quantity and quality of gametes. A more precise description of the intrinsic and environmental factors influencing reproduction would require large bimonthly samples in order to detect the degree of asynchrony in the population as well as differences in the behavior of various subpopulations. The variabilities within the samples of this study indicate that in order to obtain a value for embryo density within 20% of the true mean with 90% confidence about 70 microscopic sections or 1.33 mm^3 of each specimen would be required. Furthermore, a field sampling of 30 to 40 specimens would be required for the same statistical criteria. Data on the direct effect of incident light must await the development of techniques for long term laboratory culture of marine sponges.

Appreciation is expressed to Patricia J. Elvin for assistance with the histological preparations and in preparing this manuscript. Dr. Jefferson J. Gonor, Marine Science Center, Newport, Oregon, generously donated laboratory space and materials for this study. This work is the result of research sponsored in part by the Oregon State University Sea Grant College Program, supported by NOAA Office of Sea Grant, Department of Commerce, under Grant #04-3-158-4.

SUMMARY

1. Somatic growth and reproductive characteristics of an intertidal sponge, *Haliclona permollis*, were followed over a period of four years in a population on the central Oregon Coast.

2. Methods have been developed for estimating the instantaneous tissue temperature of sponges, calculating egg and embryo production, and measuring somatic growth rate.

3. Initiation of oogenesis during early March is best related to increases in incident light.

4. A maximum rate of oogenesis (1.5 eggs/mm^3/day) is found near the first two weeks of March, and the annual oocyte production was constant at about 44 oocytes/mm^3.

5. Temperature appears to have a secondary role in reproductive behavior but may influence sexual expression.

6. Development of embryos is related to particulate food supply in late spring.

7. Somatic growth rates are minimal from December to April and reach a maximum average of 1% per day in the fall.

LITERATURE CITED

ABERCROMBIE, M., 1941. Estimation of nuclear populations from microtomic sections. *Anat. Rec.*, **94**: 239–247.

DE LAUBENFELS, M. W., 1936. The marine and fresh-water sponges of California. *Proc. U. S. Nat. Mus.*, **81**: 1–140.

ELVIN, D. W., 1976. ,Feeding of a Dorid nudibranch, *Diaulula sandiegenesis* on the sponge, *Haliclona permollis*. *Veliger*, in press.

FELL, P. E., 1969. The involvement of nurse cells in oogenesis and embryonic development in the marine sponge, *Haliclona ecbasis*. *J. Morphol.*, **127**: 133–150.

FELL, P. E., 1970. The natural history of *Haliclona ecbasis* de Laubenfels, a siliceous sponge of California. *Pacific Sci.*, **24**: 381–386.

FELL, P. E., 1974a. Porifera. Pages 51–132 in A. C. Giese and J. S. Pearse, Eds., *Reproduction of marine invertebrates*, Volume I. Academic Press, New York.

FELL, P. E., 1974b. Diapause in the gemmules of the marine sponge, *Haliclonia loosanoffi*, with a note on the gemmules of *Haliclona oculata*. *Biol. Bull.*, **147**: 333–351.

FRY, W. G., 1970. The sponge as a population: a biometric approach. Pages 135–162 in W. G. Fry, Ed., Symposium Zoological Society London, No. 25. *Biology of the Porifera*. Academic Press, New York.

GHISELIN, M. T., 1969. The evolution of hermaphroditism among animals. *Quart. Rev. Biol.*, **44**: 189–208.

GILBERT, J. J., 1974. Field experiments on sexuality in the fresh-water sponge *Spongilla lacustris*. The control of oocyte production and the fate of unfertilized oocytes. *J. Exp. Zool.*, **188**: 165–178.

GONOR, J. J., AND A. B. THUM, 1970. Sea surface temperature and salinity conditions in 1969 at Agate Beach and Yaquina Bay, Oregon. *A technical report to the Office of*

Naval Research, data report number 39. Department of Oceanography, Oregon State University, 26 pp.

GONOR, J. J., A. B. THUM, AND D. W. ELVIN, 1970. Inshore sea surface temperature and salinity conditions at Agate Beach, Yaquina Bay, and Whale Cove, Oregon in 1970. *A technical report to the Office of Naval Research, data report number 45.* Department of Oceanography, Oregon State University, 30 pp.

HARTMAN, W. D., 1958. Natural history of the marine sponges of southern New England. *Bull. Peabody Mus. Yale,* **12**: 1–155.

HUTCHINSON, G. E., 1957. *A treatise on limnology,* Volume I. John Wiley and Sons, Inc., New York, 513 pp.

KINNE, O., 1970. Environmental factors Part I. Temperature-invertebrates. Pages 407–514 in O. Kinne, Ed., *Marine ecology.* Wiley-Interscience, London.

OLIVE, P. J. W., 1971. Ovary structure and oogenesis in *Cirratulus cirratus* (Polychaeta: Cirratullidae). *Mar. Biol.,* **8**: 243–259.

RASMONT, R., 1970. Some new aspects of the physiology of fresh-water sponges. Pages 415–422 in W. G. Fry, Ed., Symposium Zoological Society London, No. 25, *Biology of the Porifera,* Academic Press, New York.

REISWIG, H. M., 1972. The spectrum of particulate organic matter of shallow-bottom boundary waters of Jamaica. *Limnol. Oceanogr.,* **17**: 341–348.

REISWIG, H. M., 1973. Population dynamics of three Jamaican sponges. *Bull. Mar. Sci.,* **23**: 191–226.

SIMPSON, T. L., 1968. The biology of the marine sponge, *Microciona prolifera.* II. Temperature-related annual changes in functional and reproductive elements with a description of larval metamorphosis. *J. Exp. Mar. Biol. Ecol.,* **2**: 252–277.

STONE, A. R., 1970. Growth and reproduction of *Hymeniacidon perleve. J. Zool.,* **161**: 443–459.

STRICKLAND, J. D. H., AND T. R. PARSONS, 1968. A practical handbook of sea water analysis. *Fish. Res. Board Can. Bull.,* **167**: 1–311.

Reference: *Biol. Bull.*, **151**: 126–140. (August, 1976)

A SHADOW RESPONSE IN A LARVAL CRUSTACEAN

RICHARD B. FORWARD, JR.

Duke University Marine Laboratory, Beaufort, N. C. 28516, and Zoology Department, Duke University, Durham, N. C. 27706

Most benthic crustaceans have plantonic larvae which, when responding to a light stimulus, show directional swimming (phototaxis). The sign of phototaxis usually varies depending upon light intensity. In general, the pattern consists of a negative response to high intensities and a positive response to low intensities (Thorson, 1964; Forward, 1976). Recent studies, however, indicate that at high intensities the sign of phototaxis for larvae of *Rhithropanopeus harrisii* (Forward and Costlow, 1974) and *Panulirus longipes cypnus* (Ritz, 1972) does not reverse from positive to negative, but overall responsiveness is reduced. In addition, as first shown by Herrnkind's (1968) work with *Uca pugilator* larvae and more extensively with light-adapted *R. harrisii* larvae (Forward, 1974a; Forward and Costlow, 1974), responses to high and low intensities by these species are the reverse of the normal pattern. Positive phototaxis occurs at high intensities, while a negative response is seen at low intensities.

Forward (1974a) suggested that for *R. harrisii* larvae the negative phototaxis, observed upon a sudden decrease in light intensity, may function for predator avoidance. Comparable behavior is observed in sedentary animals and is defined as a *shadow reflex, i.e.*, a rapid withdrawal of exposed body parts in reaction to a shadow (Steven, 1963). Such a definition, however, is difficult to apply to a planktonic crustacean larva, which can respond with directionally-oriented movement upon an intensity decrease. *Shadow response* is perhaps a better term for such evasive movements and will be used throughout this report.

The present study is a continuation of earlier work (Forward, 1974a) providing further analysis of directional movements by *R. harrisii* larvae upon a sudden decrease in light intensity. An additional behavioral response considered herein is movement in relation to gravity. Larval crustaceans generally display a negative geotaxis in the absence of light and thereby remain swimming in the water column (Foxon, 1934; Sulkin, 1973). The sign of geotaxis can reverse to positive with changes in pressure (*e.g.*, Rice, 1964), temperature (Ott and Forward, 1976; Parker, 1902), and salinity (Hughes and Richard, 1973). An apparent positive geotaxis can result from either active downward swimming or passive sinking. Since a taxis implies a locomotor response (Fraenkel and Gunn, 1961), passive sinking in response to a stimulus cannot be considered a true positive geotaxis. Perhaps it could be more accurately described as a *sinking response*.

Thus the present study proposes the existence of a shadow response in which avoidance behavior involves oriented responses to light and gravity. It is suggested that these behaviors would be appropriate for avoiding free swimming predators on zooplankton such as ctenophores.

MATERIALS AND METHODS

Ovigerous specimens of *Rhithropanopeus harrisii* (Gould) were collected from the Neuse River in eastern North Carolina. All experiments were conducted with Stage I zoeae reared on a 12L:12D cycle at 25° C in filtered sea water at 25‰ salinity. Larvae were transferred daily to fresh sea water and were fed newly hatched *Artemia salina* nauplii. To avoid complications due to possible biological rhythms in behavior and changes during development, all experiments were begun four to six hours after the beginning of the light period on the second day after hatching. Larvae from at least three separate females were used for each experiment. The sample size reported for each experimental condition represents approximately equal numbers of larvae from each female.

Behavioral responses were monitored by placing 30–50 larvae in a lucite cuvette with a quartz entrance window, viewed horizontally by a stereomicroscope coupled to a closed circuit television system (described in detail in Forward, 1974b). The dark field microscope illumination system was interference-filtered to 802 nm (Optics Technology, Inc.; half band pass, 39 nm), a wave-length which neither alters nor induces photoresponses. The stimulus light source was a 150 w xenon arc lamp directed into a monochromator (Farrand Model F/3.5) set at 500 nm (full band pass 10 or 20 nm). Spectral purity was further regulated by a Corning No. 4–96 filter. This stimulus wavelength was chosen because a previous study of the spectral sensitivity indicated that 500 nm was the primary maximum (Forward and Costlow, 1974). Light intensity was regulated by neutral density filters and measured with a radiometer (YSI model 65). Stimulus duration was controlled by an electromagnetic shutter (Uniblitz model 225XOROX5, controlled by a model 310 drive unit). Unless otherwise stated the stimulus light was directed horizontally into the test cuvette. In some experiments, however, it was directed vertically from above. This was accomplished by interposing two prisms in the light path to elevate the beam, and then a front surface mirror was used to reflect the light down into the top of the test cuvette. To avoid problems due to the meniscus, the cuvette was carefully filled to a level parallel with its top.

Behavioral responses were recorded on video tape and analyzed as reported previously (Forward and Costlow, 1974). *Positive phototaxis* is defined as movement toward the stimulus source (±15°), while *negative phototaxis* is movement directly away (±15°). An additional behavioral response consisted of the cessation of swimming followed by downward sinking. This directly downward movement (±15°) is defined as a *sinking response.*

The speed of movement during the sinking response was determined from measurements of the distance moved in the first 0.5 second after the termination of stimulation. True sinking speeds were also measured with larvae which were anesthetized by floating a drop of propylene phenoxytol on the surface of a well slide containing the larvae. Activity usually ceased within 20 minutes, after which larvae were transferred to fresh sea water and then gently pipetted into the top of the test cuvette positioned on the microscope stage. Sinking was recorded on video tape and speeds were determined by measuring the distances moved over a 0.5 second interval. The seawater salinity for these determinations was measured with a refractometer (American Optical Company—accuracy 1.0‰) and temperature in the cuvette was monitored with a temperature probe (YSI model 420) coupled to a telethermometer (YSI model 44TD). Although the larvae were reared at 25° C, the room in which experiments were conducted was maintained at about 21° C; hence all sinking rates were performed at about this temperature. During the sinking determinations, larvae were narcotized but alive, since microscope examination indicated their hearts were beating. Greater than 95% of the animals always regained normal activity within one hour after transfer to fresh sea water. Mean speeds of movement were compared in a Student's *t*-test and significant differences tested at the five per cent level.

Conceptually, the experiments are based upon the previous finding (Forward, 1974a) that light-adapted *R. harrisii* larvae show a positive phototaxis to high intensity light and a negative response to lower intensities (Fig. 1A). Forward (1974a) suggested that these responses could participate in a shadow response during which swimming away from a potential predator occurs when its shadow falls upon a larval crustacean. The success of this behavior in avoiding predators is limited, however, because this response is only initiated upon exposure to an absolute low light intensity level, not upon a per cent decrease in intensity.

If a larva is being illuminated with light of an intensity comparable to that which occurs during the day and a shadow falls upon the animal, the light intensity could potentially decrease to either of three levels: (I) total darkness, (II) a low intensity at which negative phototaxis occurs, or (III) a higher intensity at which positive phototaxis would occur. A shadow response that functions under each of these three conditions would be most effective in predator avoidance. Thus the behavioral responses that result under these three stimulus conditions were investigated. In the experiments, the intensity of the entire stimulus beam is changed to mimic a shadow, rather than a portion of the pattern. The underlying assumption for this technique is that potential predators are much larger than an individual larva, and thus would cast a shadow over the entire animal.

The general procedure for any experiment was to light-adapt larvae under the room lights as well as a 60 w incandescent lamp for at least one hour prior to testing. Larvae were then transferred to the test cuvette which was positioned on the microscope stage. After pausing one minute in total darkness, light stimulation began. Larvae were then returned to the culture bowl and a new sample tested. Since the specific procedures varied with each experiment, these are described in detail under the Results section.

Another important consideration concerns identification of potential predators which a larva might avoid by using a shadow response. Postlarval fish occur locally in the Newport River Estuary during the summer months (Thayer, Hoss, Kjelson, Hettler and Lacroix, 1974), but gut contents indicate that they rarely feed on crab zoeae. This probably results because most zoeae are larger than the size range of organisms fed upon by postlarval fish (Kjelson, Peters, Thayer, and Johnson, 1975). Nevertheless, some small adult fish and ctenophores are reported to feed on meroplankton (Foxon, 1934). Thus feeding experiments were conducted with the small fish *Fundulus heteroclitus* and with the ctenophore *Mnemiopsis leidyi*. The latter is the most abundant ctenophore species in the Beaufort, North Carolina area and is found in great numbers during the summer months during which *R. harrisii* breeds (Schwartz and Chestnut, 1974).

The apparent optical density (O.D.) of the ctenophores along their oral-aboral

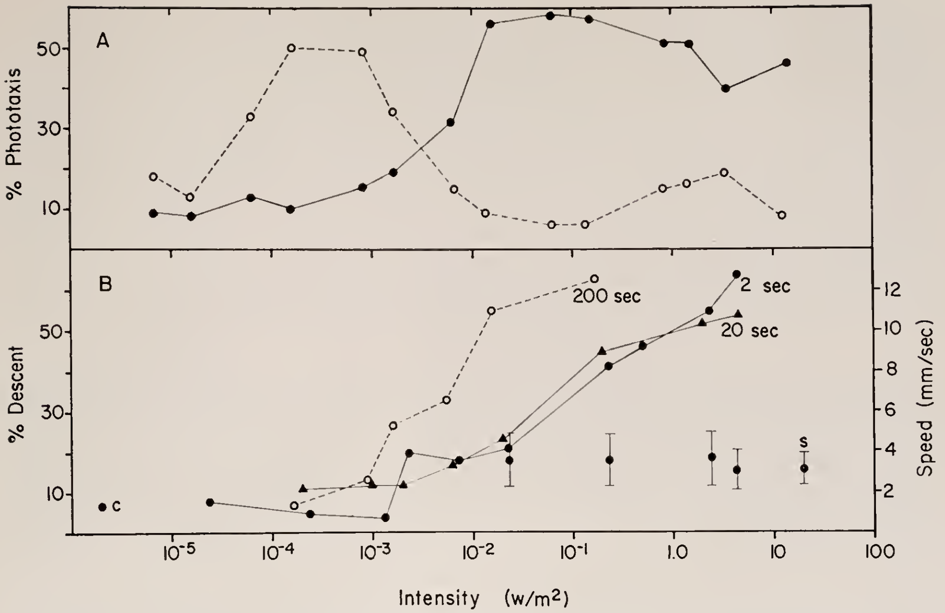


FIGURE 1. A. Per cent response (left ordinate) of positive (closed circle-solid line) and negative (open circle-dashed line) phototaxis to various stimulation intensities of 500 nm light (10 nm full band pass) (abscissa) by light adapted Stage I zoeae (replotted from Forward, 1974a). B. Percentage of light-adapted larvae showing a descent (left ordinate) upon termination of 500 nm light stimulus at different intensities (abscissa) and duration of 2 (closed circles), 20 (closed triangles) and 200 (open circles) seconds. Average sample sizes for each intensity at the different times are 56, 68, and 44, respectively. Random movement in the vertically downward direction (C-closed circle) was determined with no stimulus present. Speeds of movement (right ordinate) were recorded during the descent (closed circles) after a 2 second stimulus at the various intensities and during sinking by anesthetized animals (S). Mean speeds were plotted and vertical lines indicate the standard deviation. The average sample size for the descent and sinking speeds are 29 and 150, respectively.

and transverse axes was determined by placing freshly collected individual animals in a glass cuvette (ID 32 × 55 × 84 mm), filled with clear water of the same salinity as that in which the animals were collected. The cuvette was positioned in the sample compartment of a Cary Model 11 spectrophotometer and continuously scanned from 650 to 350 mm. The direction of the scans for all animals was descending. Plots of the apparent O.D. were made at 10 nm intervals even though the data were recorded continuously. These plots are the average of five animals within three specified size ranges, generally representing small, medium and large ctenophores as found in the Beaufort, North Carolina area.

RESULTS

Light intensity decrease from high intensity to total darkness

The general behavioral response upon extinguishing the light is a descent, during which larvae move vertically downward. This response is not observed upon turn-

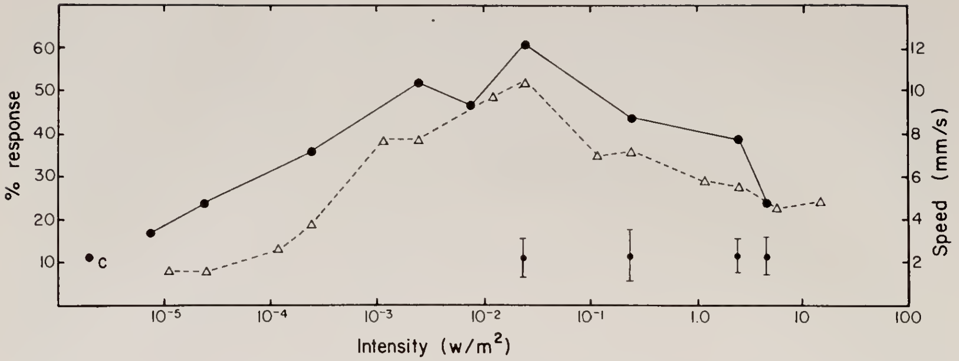


FIGURE 2. Per cent positive phototaxis (left ordinate) by dark-adapted larvae (open triangle-dashed line) upon stimulation with 500 nm light (10 nm full band pass) at different intensities (abscissa) (replotted from Forward, 1974a). The closed circles-solid line indicates per cent descent subsequent to termination of stimulation. The average sample size for each phototaxis and geotaxis point are 73 and 53, respectively. C-closed circle is the control level of descent (sample size is 73). The closed circles indicate the mean descent rates (right ordinate) at the different light intensities, and the vertical lines are the standard deviation. The average sample size is 19.

ing the light on. To establish the relationship between light intensity and this response, light-adapted larvae were stimulated at different intensity levels for 2, 20 and 200 seconds and upon extinguishing the light, the percentages of larvae showing the descent were determined. The procedure was to place larvae within the test cuvette upon the microscope stage for one minute (filtered microscope illumination only) and then stimulate three times for two seconds at 15 second intervals or two times with 30 seconds between termination and onset of stimuli for the 20- and 200-second duration stimuli. Each stimulus exposure for each larval preparation was at a different intensity. As seen in Figure 1B, the longer the stimulus duration, the greater the percentage of descending larvae at lower intensities. However, considering those intensities at which positive and negative phototaxis occur (Fig. 1A), it is apparent that the descent is only seen subsequent to stimulation at light intensities that initiate positive phototaxis.

This finding is further established by measuring per cent descent subsequent to stimulating larvae which were dark-adapted for two hours prior to testing. The technique was to pipette larvae into the cuvette under dim red 650 nm light. The preparation was placed on the microscope stage (802 nm illumination only) for one minute and then stimulated three times for two seconds each at different light intensities at 15 second intervals. Previous work (Forward, 1974a) indicated that after dark adaptation, negative phototaxis to low light intensities no longer occurred, and that the threshold intensity for positive phototaxis was lowered. In contrast, Figure 2 shows that the descent occurs in dark-adapted larvae and is greatest at those intensities which initiate the strongest positive phototactic responses.

The descent could result from either active swimming or passive sinking. To establish which of these alternatives was responsible, speeds of movement were determined during the descent subsequent to the 2-second stimulus at different intensities and for light-adapted anesthetized larvae under similar temperature (average 21.3° C) and salinity (average 25.2‰) conditions. For both light- (Fig. 1B) and dark-adapted (Fig. 2) larvae, mean descent rates are not significantly different subsequent to different stimulus intensities. Furthermore, sinking speeds for light-adapted larvae are not significantly different from those during the descent subsequent to stimulation (Fig. 1B).

Therefore, three pieces of evidence indicate that the descent results from passive sinking. The observations of the anesthetized larvae show that if they stopped swimming, they would sink. In addition, the speeds during the descent are independent of stimulus intensity even though the per cent of larvae showing the response does change (Figs. 1B and 2). Finally, light-adapted anesthetized larvae sink at rates identical to those observed during the descent (Fig. 1B). Thus the descent observed upon extinguishing the light can be termed a sinking response.

Subjective determinations were made of the minimum time duration of a light intensity decrease necessary to induce the sinking response. Larvae were stimulated vertically for one minute at an average intensity of 1.19 Wm^{-2} (500 nm–20 nm full band pass). Then, at 10-second intervals, the light was extinguished for times ranging from 10 to 90 milliseconds (as timed by the shutter control unit—accuracy 5%). The slow speed control of the video tape unit made analysis of the direction of movements upon extinguishing the light for these times impossible, so the presence or absence of a response was made subjectively. Based on eight determinations, the minimum time length that the light must be off before a positive geotaxis was always observed was 30 milliseconds or longer, while 62% of the trials showed a response at 20 milliseconds, and no responses were seen at 10 milliseconds.

Light intensity decrease from high intensity to that at which negative phototaxis occurs

Previous work demonstrates that if larvae are stimulated with high intensity light, a positive phototaxis occurs. This can be reversed to negative, if the intensity is rapidly lowered to the range of about 2×10^{-3} to 6.0×10^{-5} W/m^2 at 500 nm (Forward, 1974a). While the results from these past experiments are interesting, the procedure was somewhat contrived, since the larvae were irradiated at high intensities for only a short amount of time before the light intensity was lowered. The more realistic sequence of a longer exposure followed by a decrease in intensity initiates a sinking response followed by the negative phototaxis.

To further investigate aspects of these responses, light-adapted larvae were irradiated for different lengths of time at two intensities that induce positive phototaxis. Then the intensity was lowered to a level that should induce negative phototaxis. The time delay between the onset of the sinking response and the beginning of negative phototaxis was determined from the recorded video tapes by continuously monitoring larval position and using the video tape frame number to indicate the time. The video tape deck records at 60 frames/second. Each larval preparation was tested under only one set of stimulus conditions.

Less than 15% of the larvae began negative phototaxis within 0.33 second after the onset of the light decrease under both stimulus conditions. For the remaining larvae the delay time was greater upon longer stimulation. A direct relationship exists between the delay time plotted on a logarithmic scale and the tested stimulus times for each of the two test intensities (Fig. 3). The least squares

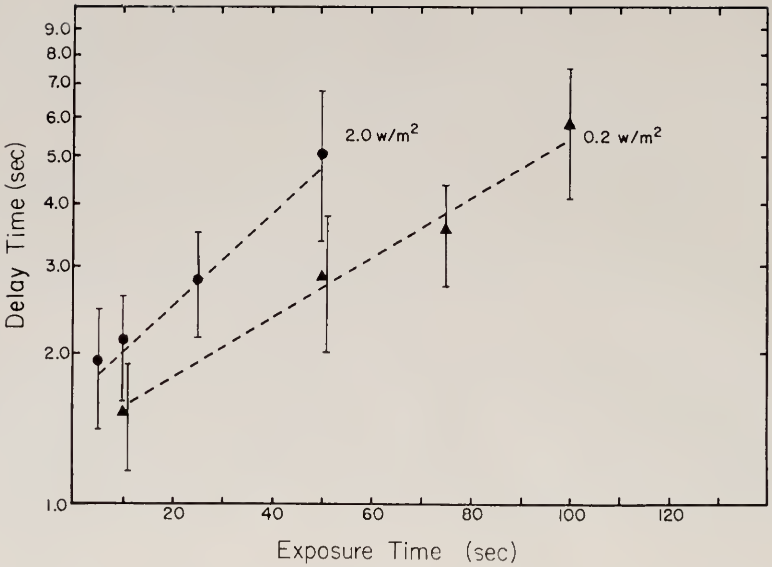


FIGURE 3. The time delay (ordinate) between the sinking response and negative phototaxis upon stimulation at 2.0 (solid circles) and 0.2 (solid triangles) W/m² 500 nm light (10 nm full band pass) for various times (abscissa) and decrease in intensity to 2.0×10^{-3} W/m². The average sample size at each time and intensity and for the determination beginning at 2.0 W/m² was 31 and at 0.2 W/m² was 21.

regression lines were calculated from the original numbers even though the mean and standard deviation are plotted in Figure 3. The slopes of the lines are highly significant ($P < 0.01$). Reciprocity is not seen for the delay time, since 10 seconds at 2.0 W/m² does not produce a similar delay time as 100 seconds at 0.2 W/m².

Light intensity decrease from high intensity to levels above those which induce negative phototaxis

If larvae are stimulated at an intensity level that induces normal positive phototaxis and the light intensity is decreased within the range that should still produce positive phototaxis, a sinking response occurs. To determine the magnitude of the intensity change necessary to produce this response and also response dependence upon irradiation time length and intensity, the following experiment was performed. Separate groups of light-adapted larvae were irradiated under three separate conditions: 10 seconds at each of two intensities of 500 nm light (20 nm full band pass), which differed by a log unit and at the lower intensity for 50 seconds. At the end of these time periods, the intensity was decreased to a specific level by adding neutral density filters (represented as delta optical density), and the percentage of larvae showing the sinking response was determined. For the 10-second exposures each larval preparation received three sets of stimuli at 30-second intervals, while for the 50-second exposures, each preparation received only two sets of stimuli.

Upon stimulation in the horizontal direction (Fig. 4A), the optical density (O.D.) that must be added to induce the first clear sinking response under the three stimulus conditions is independent of stimulus conditions as it ranges around 0.5. Similarly, under all three stimulus conditions an O.D. of 1.0 to 1.1 is needed to induce a maximal response. Increases in O.D. beyond this point do not greatly increase the response percentage.

To demonstrate that the observed sinking responses were not dependent upon stimulation from a horizontal direction, the same experiment was conducted with larvae stimulated vertically from above. These larvae were reared at 20‰, rather than 25‰, but previous unpublished data indicates that upon horizontal stimulation larval responses are identical. The results (Fig. 4B) are similar to those upon horizontal stimulation (Fig. 4A). The first clear sinking response occurs at 0.5 O.D. and the maximal responses occur at 1.0 to 1.1 O.D. and greater.

Potential predators

Small fish and ctenophores are potential pelagic predators upon larval crustaceans. *Fundulus heteroclitus* was used as the representative of small fish. In the laboratory, it readily feeds upon stage I zoeae. In subjective experiments under different directional lighting conditions in both still water and with flowing currents of different speeds, it was concluded that larvae could not escape from a "hungry" *Fundulus.* So the usefulness of the shadow reflex is questionable in avoiding large predators which visually sight and actively pursue their prey.

In the Beaufort, North Carolina, area a major predator on small zooplankton is the ctenophore *Mnemiopsis leidyi.* Although the sea nettle *Chrysaora quinquecirrha* also occurs and feeds on zooplankton, it is usually less abundant (Schwartz and Chestnut, 1972). Under laboratory conditions, if larvae are introduced into an aquarium containing *M. leidyi* (under room lights), they are readily ingested by the ctenophores and are visible in the digestive system within 30 minutes.

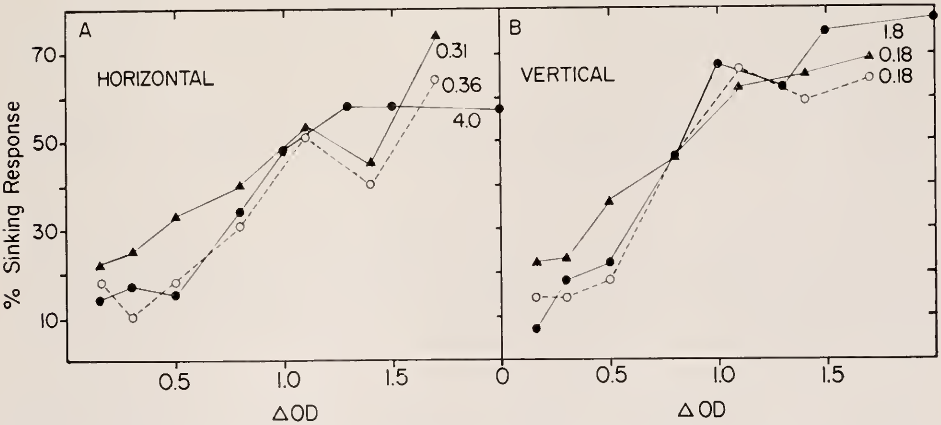


FIGURE 4. Per cent sinking response (ordinate) upon a decrease in light intensity by interposing neutral density filters in the stimulus beam path represented at delta O.D. (abscissa). Stimulation was from the horizontal (A) and the vertical (B) direction. The initial intensities (W/m^2) are shown next to the appropriate curves, and the stimulus duration for the closed and open circle curves is 10 seconds, while that for the closed triangles is 50 seconds. The average sample size for each point in A and B are 67 and 84, respectively.

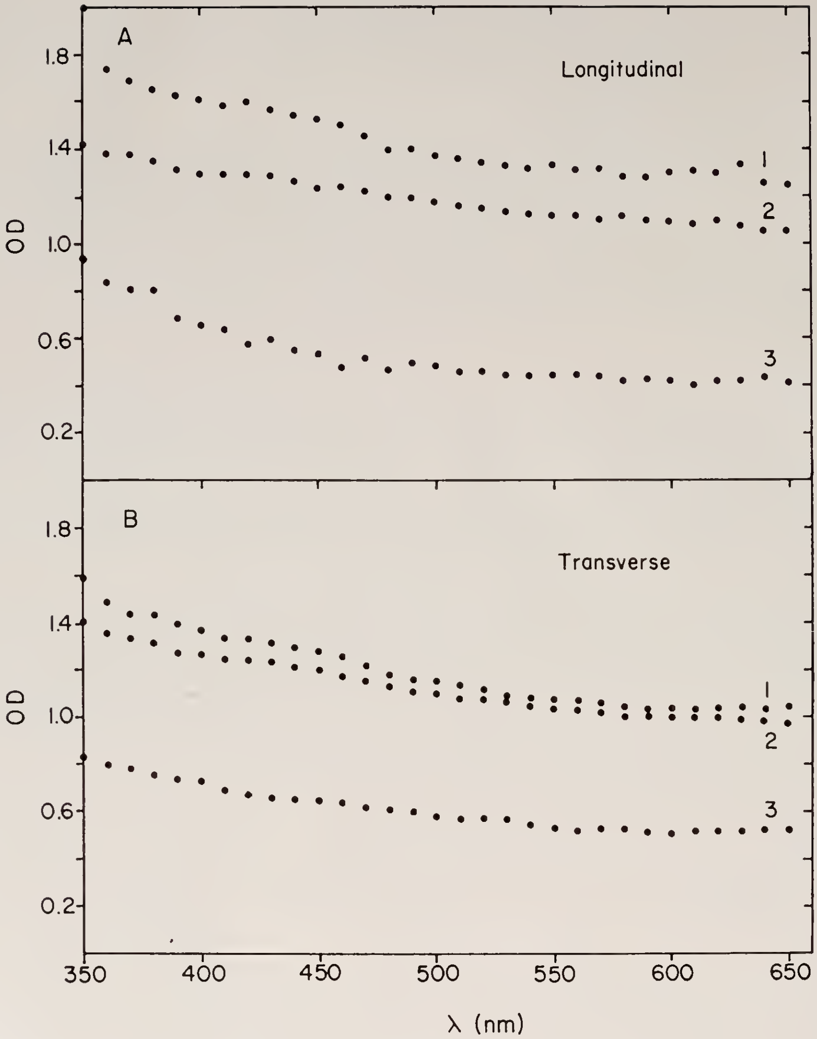


FIGURE 5. The apparent optical density (ordinate) upon longitudinal (A) and transverse (B) spectrophotometer scans from 650 to 350 nm (abscissa). The average dimensions of the three size ranges of *Mnemiopsis leidyi* tested were aboral to mouth distance 4.2 cm (1), 3.4 cm (2), 2.1 cm (3) and transverse or width distance 3.2 cm (1), 3.0 cm (2) and 1.9 cm (3). Points in each curve are averages from five animals.

Since *M. leidyi* appears to be almost transparent, a central question is whether this animal can attenuate light sufficiently to initiate the shadow response. Thus using a spectrophotometer, the apparent optical density (O.D.) due to both absorption and scattering along the transverse and longitudinal axes was measured. Since much of the attenuation results from scattering, the measured apparent O.D. may vary, depending upon the spatial relationship between the animals and the spectrophotometer light measuring system. The distance between the light exit slit and entrance into test cuvette for all measurements was 2.5 cm. Thus the measured O.D. can be considered an approximation of the attenuation by the animals.

Figure 5 shows the O.D. of whole animals in three size ranges, which arbitrarily represent small, medium, and large animals as seen in the Beaufort, North Carolina, area. Generally, the O.D. gradually increases toward shorter wavelengths with no pronounced maxima. At 500 nm both medium and large specimens of *M. leidyi* have longitudinal and transverse O.D.s greater than 1.0 and thus could initiate a maximal sinking response. Animals in the smallest size range, however, have an O.D. around 0.5, which is at the threshold level for initiating the response.

To demonstrate that a sinking response could in fact occur upon encountering a ctenophore, light-adapted larvae were stimulated vertically with 500 nm light (20 nm full band pass; average intensity, 0.73 W/m^2). During its horizontal optical path, the light passed through a rectangular leucite trough containing one *M. leidyi*. After a one-minute stimulation, the trough was pushed horizontally until the light passed through either the longitudinal or transverse body axis of the ctenophore, and the subsequent per cent sinking response was determined. Tests were run with eight *M. leidyi*, averaging 3.0 cm in width and 3.9 cm in length. According to Figure 5, these animals should have a transverse and longitudinal O.D. of about 1.1 and 1.3, respectively. The percentage of larvae moving vertically downward upon beginning the trough push was 14% ($n = 409$). The per cent sinking response upon interposing the longitudinal axis of the ctenophores was 68% ($n = 224$) and upon interposing the horizontal axis it was 57% ($n = 272$). These per cent responses agree with values plotted in Figure 4B, which shows per cent responses of about 60% for these O.D.s.

Discussion

Upon a light intensity decrease, stage I zoeae of the crab *Rhithropanopeus harrisii* show a shadow response consisting of directionally-oriented movements. Like the shadow reflex of barnacles (von Buddenbrock, 1930), the larval response only occurs upon an intensity decrease and not upon a change from total darkness to light. Although the exact behavioral responses and physiology depend upon the magnitude of the light intensity decrease, the larvae do show oriented movements under the three conditions originally proposed as necessary for a functional shadow response, *i.e.*, light intensity decrease from a high level (I) to total darkness, (II) to an intensity at which negative phototaxis occurs and (III) to a higher intensity at which positive phototaxis normally occurs.

If the initial intensity is sufficient to induce positive phototaxis and is suddenly decreased to darkness, a descent is observed for both light- and dark-adapted larvae. Comparison of the speeds of movement during the descent to those by anesthetized larvae indicates that the descent consists of passive sinking and as such is most accurately described as a sinking response. The sinking speeds by *R. harrisii* larvae are similar to those found for related species (Sulkin, 1973).

The minimum length of time that the light must be extinguished before the response is observed is 20 to 30 milliseconds. This indicates that the larvae are more sensitive to shadows than at least one barnacle species. Gwilliam (1963), in monitoring the shadow reflex of the barnacle *Mitella polymerus* by recording from the stalk nerve, found that shadows of less than 100 milliseconds in duration were not perceived by the animal. Furthermore, since the shadow response occurs in both light- and dark-adapted larvae, the response presumably can function during both day and night.

If the light intensity is suddenly reduced to a level that can induce a negative phototaxis in light-adapted larvae (Fig. 1A), most larvae show a sinking response followed by the negative phototaxis. The length of time that the sinking response continues until the negative phototaxis begins is equal to the time delay between the two responses and lengthens with increasing intensity and duration of the initial light level. As previously reported (Forward, 1974a), the negative phototaxis is not initiated by a per cent change in intensity; rather the intensity must be reduced to an absolute level. The negative phototaxis would certainly be more effective than the sinking response in avoidance, since it involves directional movement away from an area of decreased light intensity (*i.e.*, the shadow) at swimming speeds which are faster (*e.g.*, mean speed at 1.4×10^{-3} W/m^2 is 8.6 mm/sec) (Forward, 1974a) than those at which the animals sink (mean is 3.1 mm/sec). However, the significance of this absolute light level, as related to naturally occurring light intensities, remains to be determined. An alternate explanation is that negative phototaxis participates in diurnal vertical migration. Since the negative response only occurs in light-adapted zoeae, a descent at the end of the day could result from negative phototaxis to low-light intensity levels (Forward, 1974a).

At higher light intensities the maximum sinking response is initiated upon an intensity decrease equivalent to reducing the light by 1.0 to 1.1 O.D. units or greater. This amount is independent of initial light intensity, duration and direction, since identical results occur upon vertical and horizontal stimulation. This value is also higher than that found neurophysiologically by Gwilliam (1963) for the barnacle *Mitella*, for which the maximum response occurred at intensity decreases equivalent to 0.4 O.D. units and greater. The lower threshold value for initiation of the sinking response is equivalent to a reduction of about 0.5 O.D. units. This value is considerably higher than that reported from behavioral studies of shadow reflexes in adult barnacles, in which decreases equivalent to reducing the light by 0.05 to 0.12 O.D. units are needed to initiate the reflex (Forbes, Seward and Crisp, 1971). Furthermore, crustaceans are capable of perceiving much lower light intensity changes, *e.g.*, the Weber fraction for adult *Daphnia magna* at white light intensities above 40 ergs/cm^2/sec is equivalent to a 0.013 O.D. change (Ringelberg, Kasteel and Servaas, 1967).

Observations of fish feeding upon stage I zoeae suggest that larvae cannot escape large predators which visually sight and actively pursue their prey. The shadow response may be more effective in avoiding a passive predator such as the ctenophore *Mnemopsis leidyi* which, during the summer breeding season for *R. harrisii*, is an abundant pelagic predator upon local zooplankton (Schwartz and Chestnut, 1974). Like most ctenophores this species is not a powerful swimmer and captures zooplankton by using body current to draw them into its feeding apparatus (Main, 1928). From observations of gut contents, this species is reported to feed on mollusk larvae (Nelson, 1925), polychaete larvae (Main, 1928) and copepods (Nelson, 1925; Main, 1928; Bishop, 1967). Although Nelson (1925) reports that the ctenophore *Pleurobrachia* feeds on crustacean zoeae, it is not widely observed for *M. leidyi*. This is further supported by Cronin, Daiber, and Hulbert

(1962) in a study which quantitatively measured seasonal variations in zooplankton abundance in the Delaware River Estuary. During the summer of 1953 great numbers of *M. leidyi* were present and low numbers of the copepod *Acartia tonia* occurred with much higher relative numbers of crab zoeae. Cronin *et al.* (1962) speculated that *M. leidyi* was selectively feeding on the copepods, while the zoeae escaped predation due to their spines. Since *M. leidyi* readily ingests *R. harrisii* larvae in the laboratory, it is possible that the zoeae are effective in avoiding this ctenophore species under natural conditions by means of the shadow response.

In considering the plausibility of the shadow response, the ctenophore must be in a position within the water column such that it will cast a shadow upon a larva. Considering horizontal distributions, *M. leidyi* are found within estuarine-coastal water at salinities between 4–33‰ in the Chesapeake Bay (Bishop, 1972) with the largest distributions in the Beaufort, North Carolina area between about 2–23‰ (Schwartz and Chestnut, 1974). At 25° C *R. harrisii* larvae develop at salinities between 5 and 40‰ with the greatest survival at salinities 15 to 25‰ (Costlow, Bookhout and Monroe, 1966). In the Newport River Estuary, North Carolina, stage I zoeae are found between about 4 and 30‰ (Pinschmidt, 1963).

Considering vertical distribution, during the day *M. leidyi* is observed at the surface when it is calm and at shallow depths under windy conditions (Nelson, 1925). Based upon the phototactic responses by light- and dark-adapted larvae, Forward (1974a) predicted a diurnal vertical migration pattern of an ascent during the day and descent at night. This pattern is partially supported by Pinschmidt (1963) who collected stage I zoea in surface plankton tows during the day. Some larvae were also observed in bottom samples, but the overall vertical distributions were not measured throughout the day. The lighting condition necessary for the shadow response is that the initial light intensity be sufficient to induce a positive phototaxis in the larvae. This is consistent, since a positive phototaxis would contribute to the predicted vertical ascent by zoeae during the day. Therefore both specimens of *M. leidyi* and stage I zoeae are predicted to occur in similar salinity areas within estuarine areas and near the surface during the day.

The threshold for the shadow response occurs at an intensity equivalent to about a 0.5 O.D. decrease. Considering the apparent O.D./cm at 500 nm of the small size ctenophore (Fig. 5), animals larger than about 2.2 cm in length and 1.6 cm in width are predicted to have a longitudinal and transverse O.D. greater than 0.5. According to Main (1928) the adult feeding apparatus occurs in animals larger than 0.8 cm in length. This then indicates that there exists an animal size range which would not attenuate the light sufficiently to initiate the sinking response. Nevertheless, medium and large size adults are larger than this size and could cause this response (Fig. 5).

Thus, the shadow response by crustacean stage I zoeae consists of a passive sinking response which is followed by a negative phototaxis and active swimming, if the light intensity is lowered to a particular absolute level. This behavior is appropriate for avoiding zooplankton predators like ctenophores which do not actively pursue their prey but rather catch those organisms that are swept into their feeding apparatus. In addition, since ctenophores such as *M. leidyi* occur near the surface and thereby cast a downward shadow, sinking is a type of behavior that would always move a zoea away in a downward direction unless this was prevented by vertical current flows. Light intensity should continually decrease as the predator is approached. Thus, the sinking response is possibly the initial avoidance behavior which occurs some distance away from the predator, while the negative phototaxis occurs at a closer distance. The negative response would be more effective for avoidance since it involves rapid swimming directed away from the light intensity decrease. These behaviors could be used to avoid other predators, *i.e.*, coelenterate medusae such as *Chrysaora quinquecirrha* and other ctenophore species. In addition, they probably are not limited to just stage I zoeae, as they are subjectively observed in all other zoeal stages.

NOTE ADDED IN PRESS

A recent publication on the distribution and feeding preferences of *Mnemiopsis leidyi* (Burrell and van Engel, 1976) presents evidence in support of the shadow response in *Rhithropanopeus harrisii.* They report that brachyuran larvae were rarely observed in the digestive system of the ctenophore and that "zoeae of the xanthid crab *Rhithropanopeus harrisii* were present in large numbers coincident with *M. leidyi* in the summer of 1966, but were apparently not preyed on, even though they were among the smallest (0.7 mm mean length) planktonic animals." Although the authors suggest that spines may deter predation, an alternate explanation is that the zoeae avoid the ctenophores by means of the shadow response described above.

This study was supported in part by Environmental Protection Agency Grant Nos. R-801128-01-1 and R-8038383-01-0 and a regular grant from the Duke University Research Council. I thank Dr. C. G. Bookhout for providing the propylene phenoxytol; Meg Forward, Michael Latz, and Dr. Tom Fisher for their technical assistance; and Tom Cronin for his critical comments on the manuscript.

SUMMARY

1. A shadow response consisting of oriented movement to light and gravity was studied by means of a closed circuit television system for stage I zoeae from the crab *Rhithropanopeus harrisii.*

2. If larvae are irradiated at an intensity that induces positive phototaxis and the light is extingushed, both light- and dark-adapted larvae show a descent. Since this response involves passive sinking, it is termed a sinking response. The minimum time that the light must be extinguished to evoke the response is 20 to 30 milliseconds.

3. If the light intensity is reduced to a level that should induce negative phototaxis, light-adapted larvae show a sinking response followed by a negative phototaxis. The time delay between the responses is related to the initial stimulus intensity and duration.

4. The minimum decrease in intensity that induces the sinking response is equivalent to a reduction by a 0.5 O.D. neutral density filter while the maximum response occurs at optical densities of 1.0 to 1.1 and greater. These values are independent of stimulus time, intensity, and direction.

5. It is argued that these behaviors are appropriate for avoiding zooplankton predators like ctenophores which do not visually sight and actively pursue their

prey. The ctenophore *Mnemiopsis leidyi* is abundant in the Beaufort, North Carolina, area and spectrophotometric determinations of this species' apparent O.D. indicate that animals larger than a certain size attenuate the light sufficiently to evoke the shadow response.

LITERATURE CITED

Bishop, J. H., 1967. Feeding rates of the ctenophore *Mnemiopsis leidyi*. *Chesapeake Sci.*, **8**: 259–264.

Bishop, J. H., 1972. Ctenophores of the Chesapeake Bay. *Chesapeake Sci.*, **13**: S98–S100.

von Buddenbrock, W., 1930. Untersuchunger über den Schattenreflex. *Z. Vergl. Physiol.*, **13**: 164–213.

Burrell, V. C., Jr. and W. A. van Engel, 1976. Predation by and distribution of a ctenophore, *Mnemiopsis leidyi* A. Agassiz, in the New York Estuary. *Estuarine and Coastal Marine Science*, **4**: 235–242.

Costlow, J. D., Jr., C. G. Bookhout, and R. J. Monroe, 1966. Studies on the larval development of the crab *Rhithropanopeus harrisii* (Gould). I. Effect of salinity and temperature on larval development. *Physiol. Zool.*, **39**: 81–100.

Cronin, L. E., J. D. Daiber, and E. M. Hulbert, 1962. Quantitative seasonal aspects of zooplankton in the Delaware River Estuary. *Chesapeake Sci.*, **3**: 63–93.

Forbes, L., M. J. B. Seward, and D. J. Crisp, 1971. Orientation to light and the shading response in barnacles. Pages 539–558 in D. J. Crisp, Ed., *Fourth European marine biology symposium*. Cambridge University Press.

Forward, R. B., Jr., 1974a. Negative phototaxis in crustacean larvae: possible functional significance. *J. Exp. Mar. Biol. Ecol.*, **16**: 11–17.

Forward, R. B., Jr., 1974b. Phototatxis by the dinoflagellate *Gymnodinium splendens* Lebour. *J. Protozool.*, **21**: 312–315.

Forward, R. B., Jr., 1976. Light and diurnal vertical migration: photobehavior and photophysiology of plankton. Pages 157–209 in K. Smith, Ed., *Photochemical and photobiological reviews*, Volume 1. Plenum Press, New York.

Forward, R. B., Jr., and J. D. Costlow, 1974. The ontogeny of phototaxis by larvae of the crab *Rhithropanopeus harrisii*. *Mar. Biol.*, **26**: 27–33.

Foxon, G. E. H., 1934. Notes on the swimming methods and habits of certain crustacean larvae. *J. Mar. Biol. Ass. U.K.*, **19**: 829–849.

Fraenkel, G. S., and D. L. Gunn, 1961. *The orientation of animals.* Dover Publications, Inc., New York.

Gwilliam, G. F., 1963. The mechanism of the shadow reflex in Cirripedia. I. Electrical activity in the supraesophogeal ganglion and occular nerve. *Biol. Bull.*, **125**: 470–485.

Herrnkind, W. F., 1968. The breeding of *Uca pugilator* and mass rearing of the larvae with comments on the behavior of the larvae and early crab stages. *Crustaceana (Suppl.)*, **2**: 214–224.

Hughes, D. A., and J. D. Richard, 1973. Some current-directed movements of *Macrobrachium acanthurus* (Wiegmann, 1836) (Decapoda, Palaemonidae) under laboratory conditions. *Ecology*, **54**: 927–929.

Kjelson, M. A., D. S. Peters, G. W. Thayer, and G. N. Johnson, 1975. The general feeding ecology of postlarval fishes in the Newport River Estuary. *U.S. Fish Wildlife Serv. Fish. Bull.*, **73**: 137–144.

Main, R. J., 1928. Observation of the feeding mechanism of a ctenophore, *Mnemiopsis leidyi*. *Biol. Bull.*, **55**: 69–78.

Nelson, T. C., 1925. On the occurrence and food habits of ctenophores in New Jersey inland coastal waters. *Biol. Bull.*, **48**: 92–111.

Ott, F. S., and R. B. Forward, Jr., 1976. The effect of temperature upon phototaxis and geotaxis by larvae of the crab *Rhithropanopeus harrisii*. *J. Exp. Mar. Biol. Ecol.*, in press.

Parker, G. H., 1902. The reactions of copepods to various stimuli and the bearing of this on daily depth migrations. *Bull. U. S. Fish. Comm.*, **21**: 103–123.

Pinschmidt, W., Jr., 1963. Distribution of crab larvae in relation to some environmental conditions in the Newport River estuary, North Carolina. *Ph.D. Dissertation, Duke University*, 112 pp. (*Diss. Abstr.*, **24**: 11; order number 64-04735.)

Rice, A. L., 1964. Observations on the effects of changes of hydrostatic pressure on the behavior of some marine animals. *J. Mar. Biol. Ass. U. K.*, **44**: 163–175.

Ringelberg, J., J. V. Kasteel, and H. Servaas, 1967. The sensitivity of *Daphnia magna* Straus to changes in light intensity at various adaptation levels and its implication in diurnal vertical migration. *Z. Vergl. Physiol.*, **56**: 397–407.

Ritz, D. A., 1972. Behavioral response to light of the newly hatched phyllosoma larvae of *Panulirus longipes cypnus* George (Crustacean: Decapoda: Palinuridae). *J. Exp. Mar. Biol. Ecol.*, **10**: 105–114.

Schwartz, F. J., and A. F. Chestnut, 1974. Biological investigations of noxious coelenterates and ctenophores in coastal North Carolina. *North Carolina Division of Commercial and Sport Fisheries Special Scientific Report*, **27**: 1–59.

Steven, D. M., 1963. The dermal light sense. *Biol. Rev.*, **38**: 204–240.

Sulkin, S. D., 1973. Dept regulation of crab larvae in the absence of light. *J. Exp. Mar. Biol. Ecol.*, **13**: 73–82.

Thayer, G. W., D. E. Hoss, M. A. Kjelson, W. F. Hettler, Jr., and M. W. Lacroix, 1974. Biomass of zooplankton in the Newport River Estuary and the influence of postlarval fishes. *Chesapeake Sci.*, **15**: 9–16.

Thorson, G., 1964. Light as an ecological factor in the dispersal and settlement of larvae of marine bottom invertebrates. *Ophelia*, **1**: 167–208.

Reference: *Biol. Bull.*, **151**: 141–160. (August, 1976)

THE MECHANISM OF THE SHADOW REFLEX IN CIRRIPEDIA III. RHYTHMICAL PATTERNED ACTIVITY IN CENTRAL NEURONS AND ITS MODULATION BY SHADOWS[1]

G. F. GWILLIAM

Department of Biology, Reed College, Portland, Oregon 97202

One of the most noteworthy characteristics of behavior in the Cirripedia is the rhythmical nature of the so-called "fishing" operation. This consists of the extension of the body between the opercular plates, the extension of the cirri to form a net, followed by rolling up of the cirri and retraction back into the shell. The frequency of the fishing event is variable and depends in part on the species, size, temperature, oxygen concentration, pH, etc. (Southward, 1955a, 1955b, 1957, 1962, 1964; Crisp and Southward, 1961; Southward and Crisp, 1965). Some barnacles (*e.g.*, the stalked intertidal barnacle, *Pollicipes* (=*Mitella*)) do not engage in rhythmical fishing activity but simply extend the cirral net into a moving stream of water that is provided by currents in the habitat (Barnes and Reese, 1960). In addition to fishing, various degrees of "pumping" may be exhibited by barnacles. This consists of activity ranging from incomplete body extension and retraction to opening and closing the opercular plates. Blatchford (1970) has reported on another repetitive contractile process that subserves the function of circulating blood and thus substitutes for the contractile portion of the circulatory system that is lacking in Cirripedia.

Each of these activities is repeated more or less regularly within a characteristic frequency range. Such activity, of course, requires a set of controlling neural events, and Gwilliam and Bradbury (1971) reported on the occurrence of rhythmical patterned bursts of activity recorded from various nerve trunks in the isolated central nervous system of *Balanus cariosus* (Pallas). At that time an argument was presented to the effect that such patterned activity was centrally generated, and that this neural activity constituted a program that initiated and sustained the normal rhythmical behavior of the barnacle.

Another important aspect of barnacle behavior that has been recently investigated is the shadow reflex (Gwilliam, 1963, 1965; Millecchia and Gwilliam, 1972). The shadow reflex is a series of events beginning with the detection of a sudden decrease in light intensity. Following this, a sequence of neuronal events ultimately leads to the interruption of on-going activity in the barnacle, the withdrawal of the body into the protective shell, and the closure of the opercular plates. This withdrawal-closure response must involve both excitatory and inhibitory mechanisms. Upon withdrawal the nerves responsible for the contraction of muscles that cause extension must be turned off; those accomplishing withdrawal would have to be activated at the same time. Since there is no evidence of peripheral inhibition in barnacles (Hoyle and Smyth, 1963), it is likely that effects of central inhibition will be seen in the activity of motor neurons. The shadow reflex, then, may be viewed as the series of events leading to withdrawal-closure, and a simple model for the initial stages has been proposed (Millecchia and Gwilliam, 1972; Lantz and Millecchia, 1975).

---

[1] Supported by Special Postdoctoral Fellowship 1-F10-NS02342, grant no. 5-R01-NS08610 from the U.S.P.H.S. and grant no. GB-8297 from the National Science Foundation.

The above considerations suggest that it should be possible to locate central neurons that have rhythmical patterned outputs; that there should be at least two major categories of such neurons that are out of phase (see Gwilliam and Bradbury, 1971), and it should be possible to identify each group of neurons with particular muscles that are involved in extension and in withdrawal-closure. Further, the extension-related neurons should show inhibition at "light-off" while the withdrawal-related neurons should show excitation.

As reported in Gwilliam and Bradbury (1971) and previously (Gwilliam, 1968), it has proved possible to penetrate and hold a variety of cells in the ventral ganglion of the barnacle, and certain of these cells show the same activity patterns as are seen in the nerve trunks. Using such recordings as the basic approach, it has been possible to explore some of the relationships between individual cells, the motor output seen in nerve trunks, and in some cases the activity of muscles. So far it has not been possible to utilize all of the "*Tritonia* technology" introduced by Willows (1967), but the ultimate aim of this work is similar—to explain behavioral events in terms of nerve cell activity patterns and interactions.

This paper reports on the characteristics of certain of these central neurons and on their activities in relation to the behavior of the barnacle. Preliminary reports of some of this material have appeared (Gwilliam, 1973; Gwilliam and Millecchia, 1975).

Materials and Methods

Observations were made using the central nervous systems of *Balanus cariosus* (Pallas) from the Oregon coast, and general techniques were worked out on that species. This was followed by work with preparations from *Balanus hameri* (Ascanius) dredged from about 500 yards south of Langness, Isle of Man, in 10–15 fathoms. Later, more detailed observations and experiments were performed on *B. cariosus*.

Dissections were done as described previously (Gwilliam, 1965; Gwilliam and Bradbury, 1971). Three types of preparations were used in this study: the isolated central nervous system (Gwilliam and Bradbury, 1971, Fig. 1); the CNS with the last three pairs of cirri attached; and the CNS with cirri and the opercular plates with the adductor muscle intact (Fig. 1). All preparations included the median photoreceptor. Preparations were immersed in either "Instant Ocean" artificial sea water, a simpler artificial sea water, or natural sea water (the latter only at the Menai Bridge Marine Sciences Laboratory), cooled to 11°–14° C, by means of a submerged coil of polyethlene tubing through which cold water was pumped.

A bathing medium containing an excess of Mg^{2+} and a reduced amount of Ca^{2+} was used to block synaptic transmission and had the following composition: NaCl, 327 mM; KCl, 9.7 mM; $CaCl_2$, 1.0 mM (*ca* 0.1 ×); $MgCl_2$, 125.0 mM (*ca* 2.5 ×); Na_2SO_4, 28.2 mM; TRIS, 2.0 mM. This was later modified to contain only 75 mM $MgCl_2$ (*ca* 1.5 ×) with an appropriate adjustment in NaCl to maintain the same osmolarity.

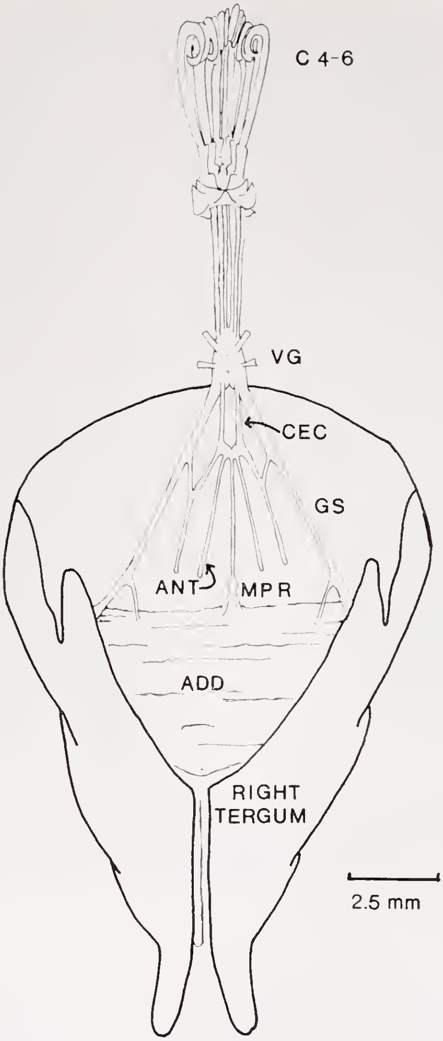


FIGURE 1. Diagram of the Cirral-CNS-Adductor Muscle preparation from *Balanus cariosus*. ANT represents antennular nerve; ADD, adductor muscle; C4-6, cirri 4 through 6; CEC, circumesophageal connective; GS, great splanchnic nerve; MPR, median photoreceptor; VG, ventral ganglion.

In view of the fact that this solution had a reduced amount of Na^+ a control was run using a medium with the normal amount of Mg^{2+} and Ca^{2+}, but with low Na^+. This solution had the following composition: NaCl, 326 mM; KCl, 9.7 mM; $CaCl_2$, 13.3 mM; $MgCl_2$, 49.0 mM; Na_2SO_4, 28.2 mM; TRIS, 97 mM. In addition controls were run with simple artificial sea water with the following composition: NaCl, 423.0 mM; KCl, 9.7 mM; $CaCl_2$, 13.3 mM; $MgCl_2$, 49.0 mM; Na_2SO_4, 28.2 mM; TRIS, 1.0 mM. The pH of these solutions was 7.6–7.8.

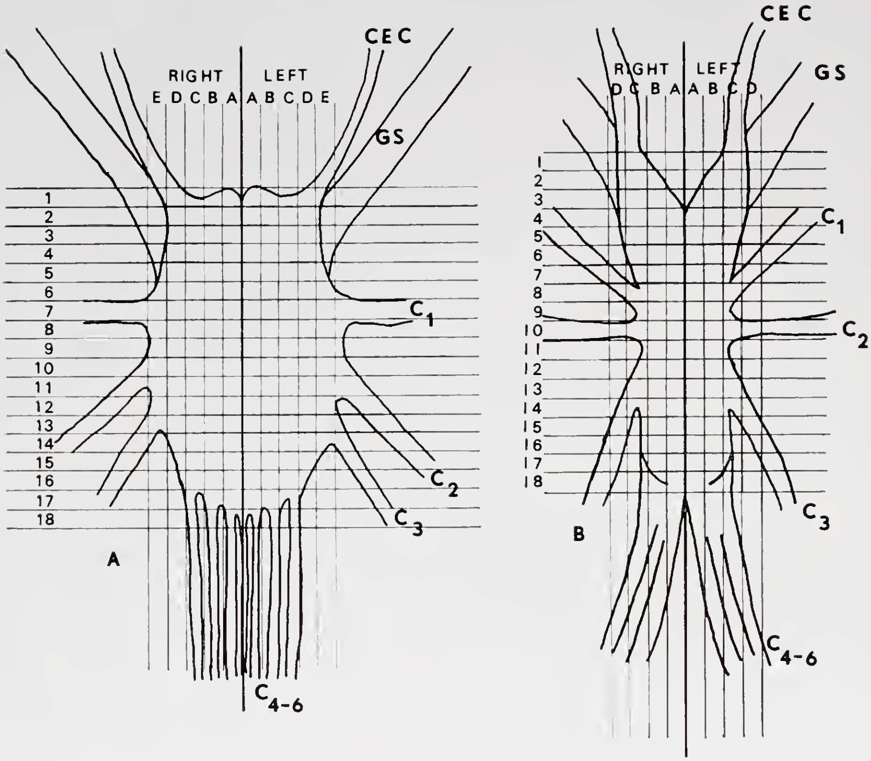


FIGURE 2. Grid reference system applied to diagram of ventral view of the ventral ganglion of each of the species of barnacles used: A, *Balanus cariosus*; B, *Balanus hameri*. The notation system designates the letter corresponding to the lateral position of the unit in question, R or L as a subscript to denote on which side of the midline the unit is located, and a number to place the unit along the anterio-posterior axis. CEC represents circumesophageal connective; C1-6, cirral nerves; GS, great splanchnic nerve.

Recording techniques were conventional, using 2M–3M KCl-filled glass micropipette electrodes of over 25 Megohms DC resistance (measured in sea water). A seawater bridge to a calomel-mercury half-cell or a Ag-AgCl pellet served as an indifferent electrode, and a similar cell was constructed to serve as the electrode holder. Neutralized input capacity amplifiers were used for intracellular recording, and one of these was designed to permit simultaneous stimulation and recording (W. P. Instruments, Camden, Conn.). External recording was accomplished with Ag–AgCl or Pt. iridium hook electrodes, or with suction electrodes. Externally recorded activity was AC amplified, and all recordings displayed on a cathode ray oscilloscope and photographed in the usual manner. Occasionally records were made on an ink-writing oscillograph.

After some experience it became apparent that certain types of cells were consistently found in the same relative positions in the ganglion. When the ganglion is viewed with transmitted light delivered *via* an image conduit embedded in the wax floor of the recording chamber, it is possible to see many neuron somata on the ventral surface and penetrate them with an electrode under visual control. A simple grid system was devised (Fig. 2) that permitted describing the positions of the recording site relative to other landmarks. In this way it soon became apparent that somata located in particular regions had particular properties, but they were not otherwise identifiable. In a few cases certain somata are identifiable from preparation to preparation because of their size and consistent location. Unfortunately, the role of these neurons in the behavior of the animals remains unknown. Obviously, this technique does not insure soma recordings, but it renders them more likely. As will be apparent below, it is possible that some recordings were from the neuropile, but the attempt was always made to restrict the depth of penetration of the electrode to the surface cells.

Penetration of cells in the intact ganglion proved virtually impossible. It was therefore necessary either to desheath the ganglion or to treat it with pronase. Both techniques proved satisfactory under most conditions. Carefully controlled application of pronase, 1–10 mg/ml, for 1.0–4.0 minutes at 10°–13° C proved to be quite reliable. On those occasions when it was desired to position two electrodes in the ganglion, desheathing was the method of choice. It was usually necessary to tap the manipulator to penetrate cells in pronase-treated ganglia, and doing this for the second cell almost always dislodged the electrode from the first cell. At irregular intervals selected nerve trunks were monitored with suction electrodes during pronase treatment and before and after desheathing to assess the effect of these procedures. There was no consistent permanent alteration of output detectable 30 minutes after the operations had been completed.

Stimulation was accomplished *via* hook or suction electrodes on one or more nerve trunks or by passing current through an intracellular electrode. A simple switching arrangement made it possible either to record from or stimulate a given nerve trunk *via* its suction electrode.

A few serial sections of the ventral ganglion of *B. cariosus* were prepared to aid in localizing neuron somata. Procion yellow and cobalt acetate injection studies have been undertaken to support the localization information gained in other ways.

RESULTS

Activity

A review of barnacle functional morphology may facilitate understanding of some of the matters discussed below The following information is derived from unpublished observations, from Gutmann (1960), Crisp and Southward (1961), Bullock and Horridge (1965), and Gwilliam and Bradbury (1971).

The nervous system. The central nervous system of balanoid cirripeds consists of a small, bilobed supra-esophageal ganglion, connected to the ventral ganglion *via* the circumesophageal connectives. The ventral ganglion is composed of the fusion of several primitively separated segmental ganglia (seen in, *e.g., Mitella*) into one mass that shows little superficial trace of segmental structure (Batham, 1944; Cornwall, 1953; Bullock and Horridge, 1965). The topography of the principal nerve trunks is adequately described in various sources, including Gwilliam and Bradbury (1971), but no detailed histological description of the *Balanus* central nervous system has been published to date. Examination of sections of the ventral ganglion shows nerve cell somata located around the periphery, mainly on the ventral surface, with central neuropile. Cell sizes are variable, ranging from 80 μm to around 5 μm. Cells in locations that correspond to positions of the bursters to be described are mostly in a size range of 20–30 μm, and it is assumed that such is the size range of most of the cells penetrated.

Behavior. The action of various muscles during the main behavioral act of a barnacle—fishing or "beating"—has been described by Gutmann (1960) and by Crisp and Southward (1961). The operculum is connected to the shell by means of a flexible chitinized membrane and can move in all directions. Upward movement is brought about by fluid pressure within the membrane sinus and is limited by the elasticity of the membrane and the degree of relaxation of the three pairs of opercular depressor muscles. The prominent adductor muscle bridges the two scuta, and its contraction closes the valves. According to Crisp and Southward (1961), the adductor is involved in rhythmical activity and does not act like a bivalve mollusc adductor in holding the valves closed when the barnacle is out of water. Hoyle and Smith (1963), however, describe a possible mechanism permitting long term contraction without fatigue in this muscle.

The six pairs of cirri differ in form and function. The first three pairs are short and stout, the other three pairs are long and slender. Basal musculature is similar in all cirri, but the longer cirri have only flexor muscles in the rami which roll them up for withdrawal into the mantle cavity. They are extended by the movement of body fluid forced into them by muscles in the prosoma.

While it is only possible to infer detailed muscle action from the movement (or lack of it) of the body, it seems most probable that opening of the operculum occurs in the following way (Gutmann, 1960) : in the closed position, the adductor muscle and the rostral scutal and carinal tergal depressor muscles are contracted. The lateral scutal depressors are relaxed, as are the basal mantle muscles. Under these conditions the operculum tends to be rather flat. When opening is taking place, the basal mantle muscles contract, forcing fluid from the base into the subtergal sinus and surrounding tissue; the tergal depressors and the rostral scutal depressors are relaxing, and the effect is to elevate the terga due to the increased pressure in the sub-tergal sinus. As the terga are thrust up, the scuta are carried along, because they are connected to the terga. The adductor muscle relaxes, and the lateral scutal depressors are contracted. This pulls down the lateral edges of the scuta at their widest point and effects opening of the opercular plates. The plates may be opened and shut in this extended condition by antagonistic action of the adductor and the lateral scutal depressors. The sequence would be: a) tergal and rostral scutal depressors relax; b), simultaneously the basal mantle muscles contract; c), the adductor scutorum muscle relaxes; and d), the lateral scutal depressors contract. This sequence gets the operculum open so the body may be extended.

While the animal is closed, all the dorsal musculature, the main longitudinal ventral body musculature, the oral cone depressors, and the adductor are contracted. Just prior to, and continuing coincident with valve opening, paired muscles attached to the scuta and the lateral body exoskeleton (numbered 1, 2, 3, and 8 by Gutmann, 1960) contract as the dorsal and ventral musculature of the body relaxes. This presses the body up into the angle of the valves, causes fluid to move from the prosoma to the thorax and extends the thorax and the cirri. As the valves open, the body is thrust out through the opening, and the last three pairs of cirri unroll to form the net. Another muscle (designated no. 6 by Gutmann, 1960) elevates the oral cone. Reversal of these actions would result in retraction.

Innervation of muscles. The distribution of nerves to muscles is not well known, but some nerve trunks have been traced with the aid of methylene blue staining and electrical stimulation. These observations have established that the great splanchnic nerve supplies motor fibers to the adductor muscle, the oral retractors, the lateral muscles designated no. 8 by Gutmann (1960), and some other muscles associated with the mantle cavity. The antennular nerve serves both pairs of scutal depressors and probably the tergal depressors. The cirral and paracirral nerves send fibers not only to the cirri but to body musculature. The nerve designated as the mid-dorsal nerve in Gwilliam and Bradbury (1971) has been traced to transverse (circular) muscles dorsal to the nervous system but ventral to the gut. Contraction of these muscles causes increased pressure and movement of body fluid and is probably involved in extension of the body.

Neurophysiology

Figure 2 consists of diagrams of a ventral view of the ganglion of each of the species used with the grid system appropriate to each. The ganglia are morphologically similar, and most generalizations to be reported apply to both species. The shadow reflex in *B. hameri* is not as well developed as it is in *B. cariosus*, but in other respects I am unable to detect major differences. The neurons that have been studied to date are located on the ventral surface and lateral aspects of the ganglia. Those on the dorsal surface have not been systematically examined.

A general search of the ganglion reveals many units that are silent and cannot be driven with application of up to 15 na of depolarizing current. Others are silent but can be driven, still others respond to "light-off" by firing one to many spikes, while others are spontaneously active. The spontaneously active units may be pacemaker-like, irregularly active and obviously receiving considerable synaptic input, or produce quite regular patterned output (Fig. 3). This latter will be referred to as "bursting" and the units called "bursters" because of the similarity of this output to cells so designated in other invertebrates (*e.g.*, Strumwasser, 1965). Units other than the bursters have not yet been linked to any behavioral event other than the coincidence of an "off response" seen in some. The bursters, on the other hand, may be correlated to behavioral events, and a description of their properties and activities forms the main part of this paper.

Identification of types of units. Systematic penetration of large numbers of somata on the ventral surface of the barnacle ventral ganglion has revealed a pattern of distribution of two general categories of bursters: the Midline Inhibited Bursters (MIB) and the Lateral Excited Bursters (LEB). The MIBs are usually found along the midline and are inhibited at light-off (Fig. 3A, B), while the LEBs are usually found laterally and are excited at light-off (Fig. 3C). In *B. hameri* the rather tenuous nature of the shadow reflex in the isolated CNS forces a greater reliance on position of the unit for identification, but other characteristics of the bursting patterns usually support the positional recognition. LEBs in general have shorter bursts and shorter interspike intervals (higher frequency) earlier in the

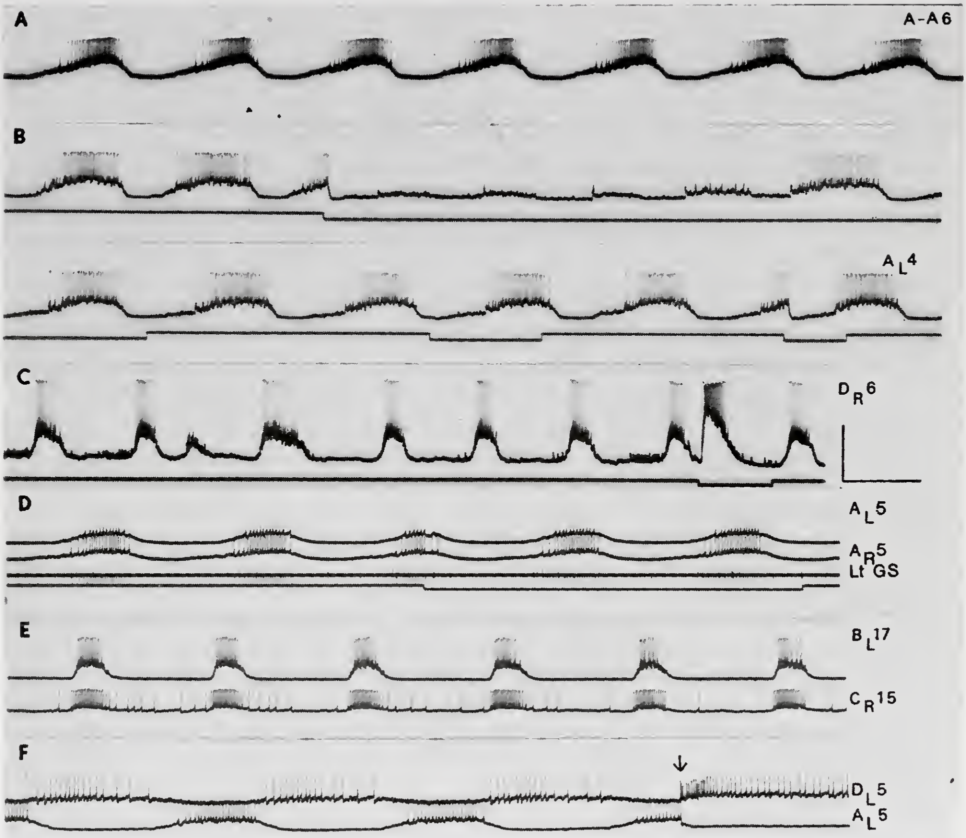


FIGURE 3. Examples of units from *B. cariosus* with regular, patterned output. Grid reference locations noted with each record. A, B. Characteristic Midline Inhibited Burster (MIB). In B, note the effect of "light-off" (lower trace). If off occurs during a burst, that burst is inhibited and subsequent bursts reset. If off occurs during the silent phase there is no significant effect, and "on" causes no change whenever it occurs. C. Lateral Excited Burster (LEB) showing the effect of "light-off" (lower trace). D, E. Illustrates the fact that MIBs are in phase with other MIBs and LEBs are in phase with LEBs. F. Simultaneous recording from a midline burster (lower trace) and a lateral burster (upper trace) to show reciprocity. Calibration is A, B, D, E, F, vertical, 50 mV, horizontal, 4 seconds; C, 25 mV, 4 seconds. Lt GS represents left great splanchnic. Unlabeled lower traces monitor light; downward deflection is "off." Stimulus was delivered to right great splanchnic at arrow in F.

burst than the MIBs (compare Fig. 3B, C, and see Fig. 3E). Membrane potentials are difficult to determine with certainty because of the frequent oscillations. In cells that become silent, however, LEBs have higher membrane potentials (40–50 mV) than MIBs (30–40 mV), but there is some overlap. Action potential amplitude is variable, but does not overshoot zero potential.

The duration of inhibition or excitation depends upon the immediate history of the preparation. If a number of decreased illumination events have occurred the effect on the unit appears to be phasic (1–4 sec); if a period of adaptation lasting

10 minutes or more has preceded the shadow, the effect is long-lasting (>15 sec). In short, accommodation is seen, as described (Gwilliam, 1965) for the shadow reflex. This corresponds to the behavior of the intact animal.

An occasional inhibited burster is found laterally, and on one or two occasions a cell that is both inhibited and excited, depending on when in the burst cycle light-off occurs, has been seen. In general, however, the populations are distinct even though there is considerable variability in details of the patterned output each produces.

Temporal characteristics of bursters. The fact that these two kinds of cells respond oppositely to "light-off" suggests that their bursting patterns would be reciprocal. This is indeed the case as shown in Figure 3F. I have never seen two inhibited bursters 180° out of phase, although they may be out by some lesser amount (Fig. 5A). The length of the burst, the number of spikes per burst, and the frequency of the bursts, can be quite variable and are different in the two species. Interburst spikes are frequently seen (Fig. 3E). They may be the result of some slight injury to the cell, or they may be part of the normal pattern (Cf. Burrows, 1974, Fig. 1). Either explanation may be applicable under certain conditions. There is no reason to assume that muscles acting on a hydrostatic skeleton would go to zero tension. In fact, quite the opposite is likely as a means of maintaining turgor.

Correlation with behavior. The burst cycle length in each species falls within the cycle length of fishing and/or pumping behavior in the intact animals as they are observed at comparable temperatures (11°–17° C) in the laboratory (Gwilliam and Bradbury, 1971, for *B. cariosus*). Further, long term recording from a single MIB corresponds to a behavior that is characteristic of barnacles. In intact animals, fishing or related behavior will be initiated, apparently spontaneously, and will also cease for no obvious reason. This behavior occurs at irregular intervals, and the animal may remain closed for considerable periods of time. Mid-line bursters in an isolated CNS "behave" in a manner that reflects the behavior of the intact animal (Fig. 4). This demonstrates the persistent nature of the patterned output of such cells and the lack of dependence on any extrinsic timing cues. No evidence of a circadian rhythm in the behavior has been noted (Cf. Sommer, 1972).

Further indication of the relationship of these cells to activity in the intact animal may be obtained in the following ways. First a moment's reflection suggests that the MIBs are at least temporally related to *extension* of the barnacle (accomplished by muscles acting on the hydrostatic skeleton) because they are inhibited at "off" which would be a necessary event at withdrawal induced by a shadow. The reverse is true of the LEBs, and so they are probably involved in *retraction.* Secondly, it is possible to record from single cells and the adductor muscle simultaneously. As shown previously (Gwilliam and Bradbury, 1971), the adductor muscle junction potentials may also display a rhythm of the same cycle length as the patterned activity. From observations of adductor muscle activity in a pumping barnacle, it can be ascertained that the adductor is relaxed and being extended during body extension, and it contracts during body withdrawal. If one now examines the relationship between a burster and the adductor junction potentials, a conclusion about the function of the bursters may be drawn (Fig. 5B, C). Thirdly, ascertaining the nerve through which the axons of the bursters exit from

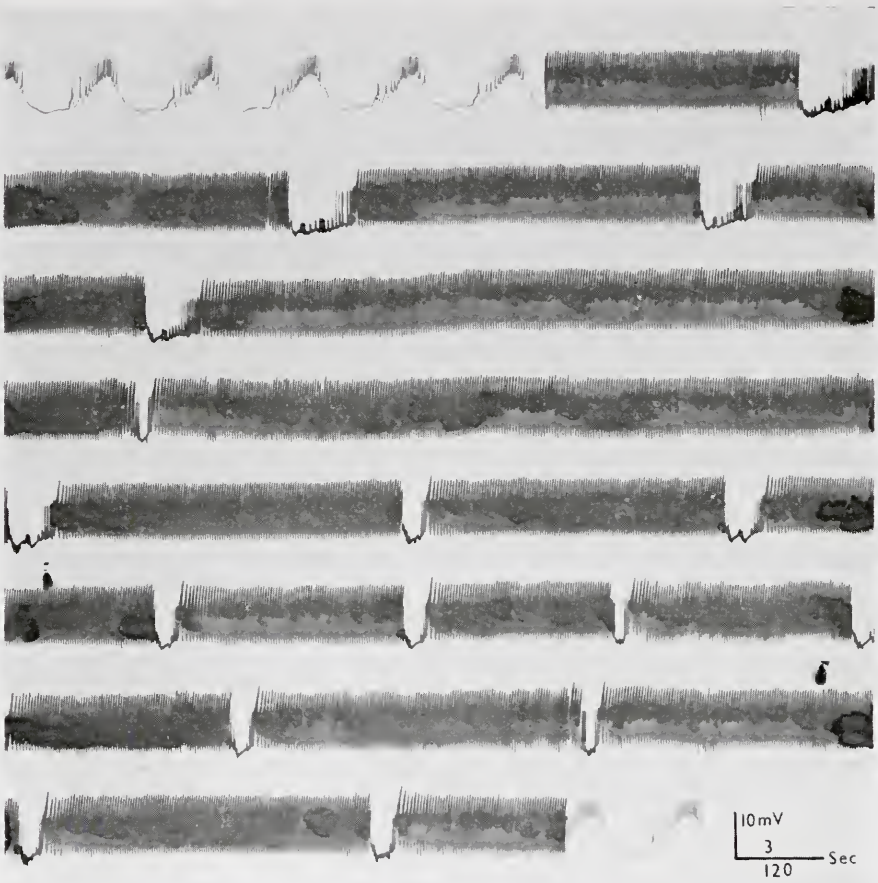


FIGURE 4. Approximately 2 hours and 20 minutes from a continuous record of 20 hours and 25 minutes duration from a Midline Inhibited Burster from *B. cariosus*. This portion is from 8:00 to 10:20 PM. The total run was from 3:15 PM one day to 11:40 AM the next day. Chart speed was increased at beginning and end of record to show characteristic normal burst pattern. Calibration is as noted. Horizontal bar is 3 seconds for beginning and end of record, 120 seconds for remainder.

the ganglion and then tracing the nerve to the muscle it serves can be useful (Fig. 5D; Fig. 6). In cases where the nerve trunk serves many muscles of different function (such as the great splanchnic), this procedure is less useful, but in some cases the nerve distribution is limited and permits the functional conclusion to be drawn. Fourthly, the phase of a burster with the patterned output of various nerves can be useful in some instances. For example, knowing how a particular burst externally recorded relates to the adductor, and knowing how a burster intracellularly recorded relates to the nerve, will permit assigning a role (extension or contraction) to the burster.

Examples of the last three kinds of evidence are shown in Figure 5, B–F, and bear out the conclusion based on the response to shadows. The fact that some of these cells have a demonstrable 1:1 coincidence with a fiber in a particular nerve and can be antidromically stimulated *via* the nerve is good evidence that the nerve recorded from contains the axon of the penetrated unit, and supports the idea that the bursters are motor cells. One unit illustrated (Fig. 5D) is a lateral burster,

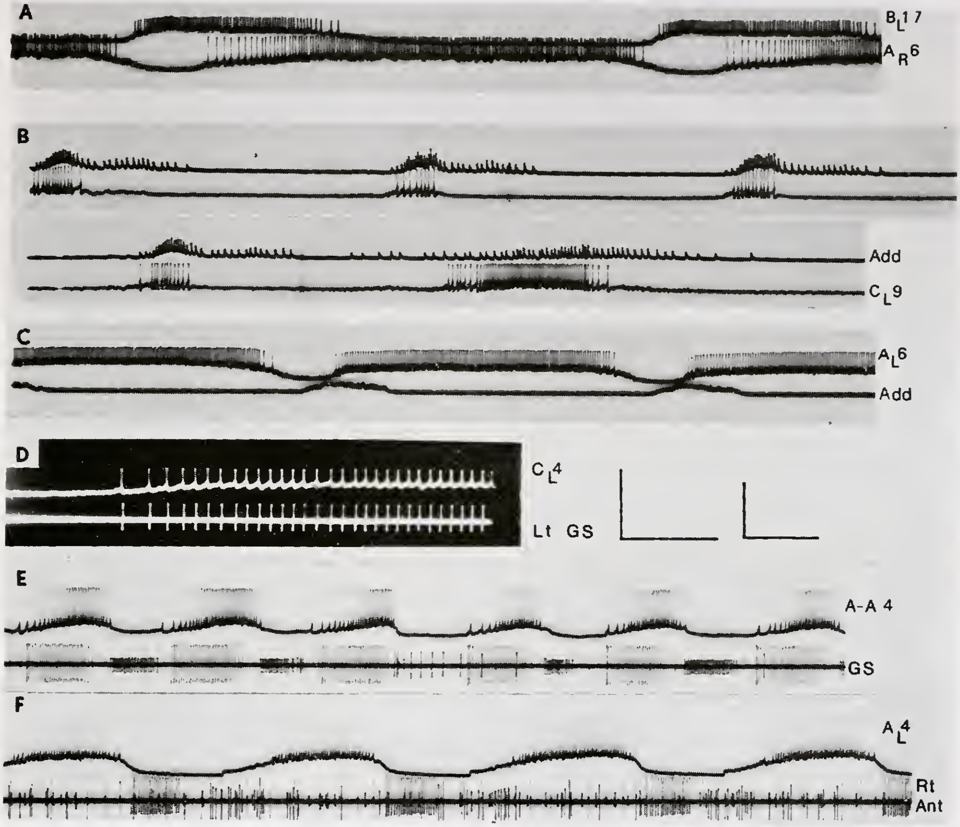


FIGURE 5. Temporal relationships: A–D, *B. hameri*; E. F, *B. cariosus*. A. Simultaneous recording from two inhibited bursters, one well caudad of the other (B_L 17, upper trace) showing overlapping patterns that would be expected of a posteriorly originating, anteriorly progressing wave of muscle contraction driven by these neurons. B. Simultaneous recording from an adductor muscle fiber (upper trace) and a lateral burster. Note that they are in phase as would be predicted for units activated at "off" that function in the withdrawal-closure response. C. The same for a midline burster (upper trace) and the adductor muscle, these two being out of phase. D. Intracellular recording from a lateral burster (upper trace) and an external recording from a branch of the ipsilateral great splanchnic nerve. The 1:1 correspondence indicates that the axon of C_L4 is in that branch of the great splanchnic. E. Intracellular recording from a midline burster (upper trace) and an external recording from the great splanchnic. Note that of the two obvious bursters in the great splanchnic, one is in phase, the other out of phase with A–A4. F. Similar type of recording with the right antennular nerve. Calibration is A–D, left calibration figure. In A and C, vertical bar is 60 mV, horizontal, 4 seconds; B, 7.5 mV (upper trace), 75 mV (lower trace), 4 seconds; D, 60 mV, 2 seconds. The right calibration figure applies to E and F. The vertical bar represents 5 mV; horizontal bar, 2 seconds.

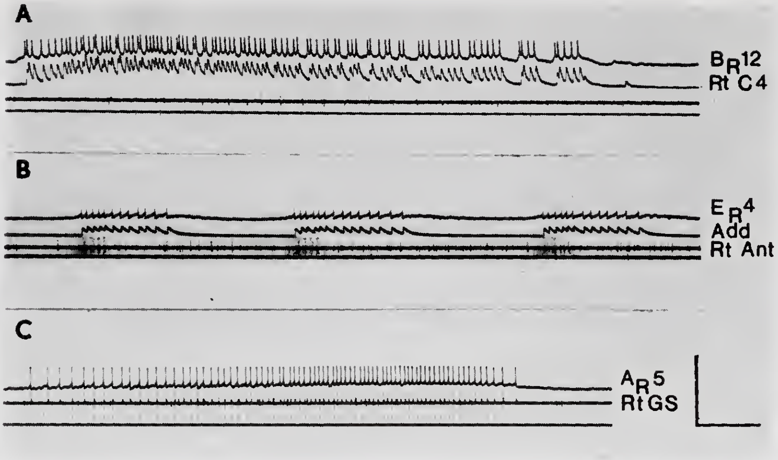


FIGURE 6. *B. cariosus.* A. Posteriorly located lateral burster (upper trace) and the muscle it drives at the base of the left fourth cirrus. B. Anteriorly located lateral burster (upper trace) and the adductor muscle fiber it innervates. C. A midline burster (upper trace) with the branch of the great splanchnic containing its axon. Rt Ant represents right antennular nerve; Rt GS, right great splanchnic nerve. Unlabeled bottom traces monitor light. Calibration is 50 mV, 0.5 seconds.

and its axon apparently exists through a branch of the great splanchnic nerve that innervates the adductor muscle. Similar records have been obtained from the midline bursters, the mid-dorsal nerve, and a branch of the great splanchnic nerve which can be traced to muscles important in the hydrostatic extension of the trunk and cirri (Fig. 6C).

Final proof that certain of the cells described as "bursters" are in fact motor cells has been obtained in preparations that include three pairs of cirri (those that make up the "fishing net") and/or the scutal adductor muscle. It has been possible to penetrate and identify (by their electrical properties) units in the posterior lateral aspect of the ganglion as LEBs and by passing current fire the cell and observe localized contractions in a single cirral ramus coincident with that firing. On a few occasions penetrations of a burster and the muscle fiber it drives have been accomplished (Fig. 6A, B) both in cirral muscles and in the adductor. Such units are identifiable as lateral excited bursters. It has not been possible to make such positive identification of MIBs, because the hydrostatic muscles that lead to extension are not as easily prepared as the "flexors." One-to-one correspondence between a midline burster and the motor supply to hydrostatic extensor muscles, however, has been obtained (Fig. 6C). This particular cell was successfully injected with procion yellow which confirms the mid-ventral location, the soma recording site, and the axon going in the direction of the great splanchnic nerve.

Origin of the bursting pattern. The next question that arises is one concerning the relationship of the bursters to each other. Cells, such as these, that have reciprocal patterns may well be thought of as being mutually inhibitory. This is not true of these units. Changing the frequency or introducing spikes during the silent period of one burster has no effect on any other burster from which I have been able to record. Cells other than bursters have been driven as well, and to date no clear influence of any other unit—other than the photoreceptor—has been discovered. Gross stimulation of cirral nerves and the great splanchnic nerve results in the same inhibition-excitation phenomenon seen when a shadow is cast, but those are the only influences seen to date (Fig. 3F). Attempts to start a silent burster by stimulation of nerve trunks have not been successful. It seems clear that whatever controls the bursting pattern exerts its influence on each burster and does not rely on these cells influencing each other, *i.e.*, there is no evidence that the bursters are coupled in any

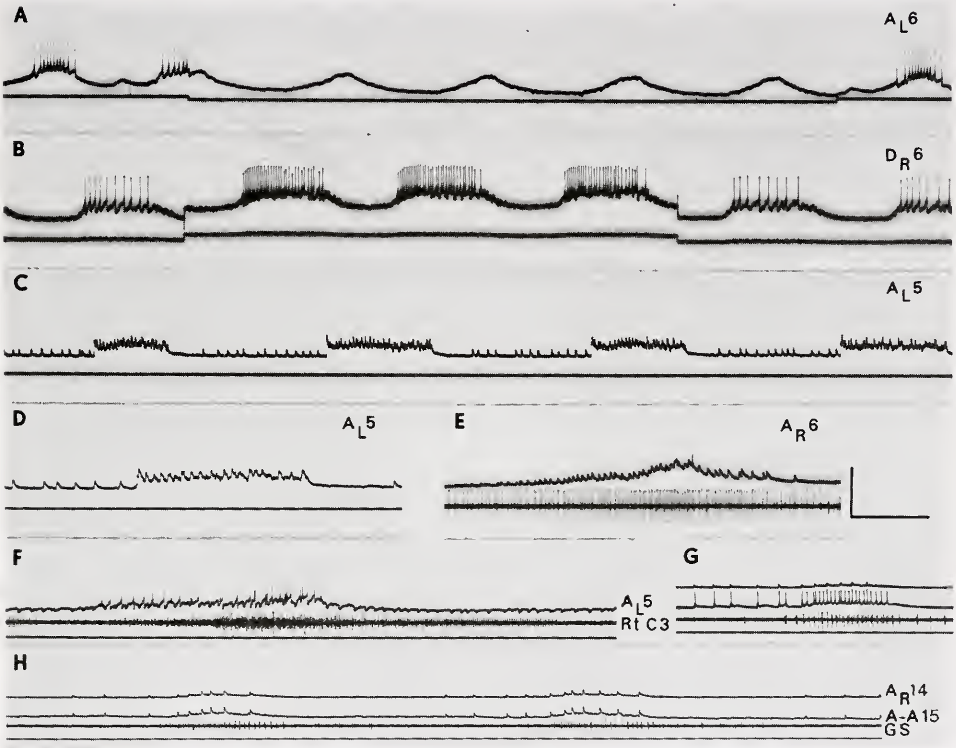


FIGURE 7. *B. cariosus:* A. Midline burster subjected to hyperpolarizing current (monitored on lower trace) sufficient to completely block spiking. Note occurrence of rhythmical membrane depolarization without significant re-setting. B. Depolarizing current applied to a lateral burster, again showing no effect on burst period. C. Midline burster showing post-synaptic potentials in the absence of spiking that frequently occurs spontaneously. EPSPs are prominent, and some indication of IPSPs may be seen in D, a record from the same cell at higher film speed. E. A midline burster subjected to hyperpolarizing current clearly showing EPSPs. F. Midline burster at higher gain to illustrate IPSPs especially during interburst interval. G. Simultaneous recording from neighboring cirral motoneurons (classed as LEBs) to illustrate the mechanism of synchronization. A_R 14 is spontaneously nonspiking and shows some EPSP coincidence with spikes in A–A15. H. A–A15 was hyperpolarized to suppress spiking and now shows 1:1 EPSP coincidence implicating a single presynaptic unit driving both cells. Rt C3 represents right 3rd cirral nerve; GS, great splanchnic. Calibration is A-C, E, G, 40 mV, 2 seconds; D. H., 40 mV, 0.8 seconds; F, 14 mV, 2 seconds.

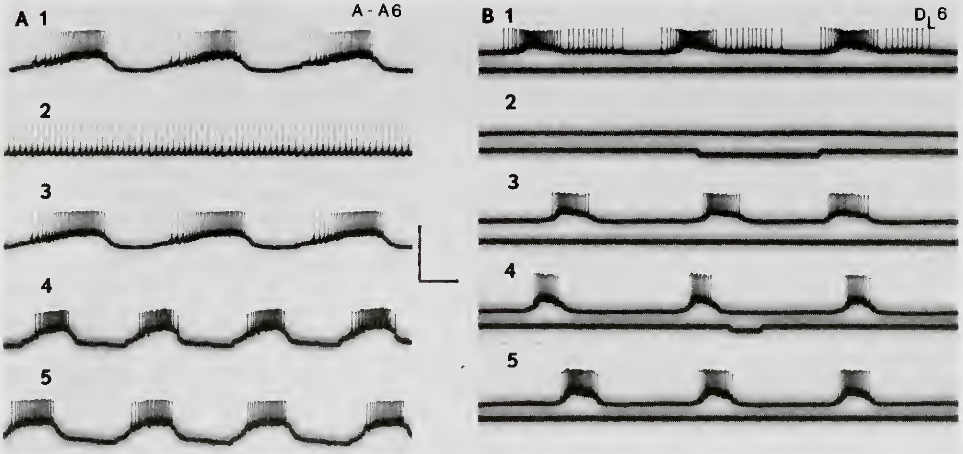


FIGURE 8. *B. cariosus:* Origin of patterning in bursters. A 1–5, Midline Inhibited Burster; B 1–5, Lateral Excited Burster. A 1. Spontaneous pattern in Instant Ocean artificial sea water. A 2. 15 minutes after changing to high magnesium, low calcium medium. A 3. 15 minutes after return to Instant Ocean. A 4. Several hours later, pictures taken 15 minutes after change to simple artificial sea water. A 5. 15 minutes after change to low sodium control medium. B 1. Spontaneous pattern in Instant Ocean. B 2. Two minutes after changing to high magnesium, low calcium medium. B 3. Twenty two minutes after return to Instant Ocean. B 4. Two minutes after changing to low sodium control medium. B 5. Two minutes after return to Instant Ocean. Calibration is vertical, 50 mV, horizontal, 2 seconds. *NOTE:* The change in burst frequency and form as seen between A 3 and A 4, A 5 and between B 1 and B 3, B 5 is not uncommon over a period of time. Such changes cannot be ascribed to experimental manipulations.

direct way (Mulloney and Selverston, 1974a, b; Selverston and Mulloney, 1974; Hoyle, 1964).

Examination of records of bursting patterns under various conditions demonstrates that there is a great deal of synaptic input that serves either to modulate an endogenous pattern, or to create the pattern, or both. On occasion, spontaneous depolarization occurs that does not lead to spiking. Such records sometimes show depolarizing PSPs on the rising phase and hyperpolarizing PSPs on the falling phase. This phenomenon suggests that the burst is driven, but it is also possible that these are simply modulating inputs.

In order to determine if the bursts are endogenously generated, two tests were performed: a) injecting depolarizing and hyperpolarizing current and observing the effect on burst patterns; and b) using high Mg^{2+}, low Ca^{2+} perfusing medium to block transmitter release and observing the effect on bursts.

Examination of records of impaled bursters when they are not spiking often shows trains of EPSPs that occur at bursting frequency (Fig. 7C, D). Hyperpolarizing the cell will suppress spiking and often permit these post-synatic potentials to be seen (Fig. 7A, E). Membrane oscillations occur at the expected interval, *i.e.*, no re-setting occurs. This is also true if the cell is depolarized. The latter leads to higher frequency firing and slightly longer bursts, but the periodicity of the bursts remains essentially unchanged (Fig. 7B). These facts suggest that the burst is driven by synaptic input.

Further evidence to support this comes from observing the effect of changing the medium to one containing high magnesium and low calcium which is known to block transmitter release in other systems (Rubin, 1970; Gainer, 1972). While it is difficult to hold cells long enough to accomplish a complete change of the bathing medium to the high Mg^{2+}, low Ca^{2+} solution and then return it to artificial sea water, some successful manipulations have been carried out. Under the experimental medium, all signs of the bursting pattern disappear and the baseline becomes smooth, indicating no synaptic input. In some MIB cells, the cell begins spiking at a regular frequency. In either case the bursting pattern returns when the medium is changed back to sea water (Fig. 8A). Some MIB cells and all of the LEB cells successfully tested simply become silent under high Mg^{2+}, low Ca^{2+} treatment (Fig. 8B).

Synchronization. Observations of cirral contractions show that most of the muscles of a cirrus contract simultaneously. This means that the motoneurons driving the muscles must be synchronized in some fashion, and the fact that members of a given class of bursters are in phase is indicative of some synchronization mechanism. The most convenient way to examine this is to impale LEBs close together in the posterior part of the ganglion where it has been shown LEBs are cirral motoneurons. Cobalt back-filling *via* a cirral nerve establishes that cell bodies with axons in that nerve are clustered around the root. By impaling cells lying physically very close together at the root, it is sometimes possible to demonstrate that they have a common presynaptic drive unit (Fig. 7G, H) that serves to synchronize the motoneurons.

Thus, it seems clear that the patterned output of the bursters is driven by input from other cells. One possibility is that there is a minimum of two presynaptic cells, both excitatory to the bursters, and mutually inhibitory. Such a system (two presynaptic units) is required because of the opposite actions induced by a shadow and normal pattern reciprocity. A single unit, having both excitatory and inhibitory actions on *two* follower cells, however, has been reported in *Aplysia* (Kandel, Frazier and Coggeshall, 1967), so it is not unreasonable to postulate a similar mechanism here for the presumed second-order cells in the shadow reflex pathway (Millecchia and Gwilliam, 1972).

There is, however, one clear effect on the bursters, and that is the inhibition-excitation that occurs at "light-off." Suitable records illustrate that inhibition takes place on a presynaptic unit, because this event *does* reset the burst pattern (Fig. 3B) and may act directly on the unit or units that generate the patterning.

As yet no candidates for the "oscillator" have surfaced. No oscillator neurons having the characteristics described by Mendelsohn (1971) or Pearson and Fourtner (1975) have been seen, nor have any units that have a direct affect on burst pattern. It can easily be demonstrated that whatever the burst generating mechanism is, it is not restricted to the supra-esophageal ganglion. Removal of the supra-esophageal ganglion has no effect on patterned output from the ventral CNS once the injury effect has terminated.

Discussion

There is still a great deal that may be learned concerning the generation of patterned output and its relation to behavior in the barnacle preparation. This

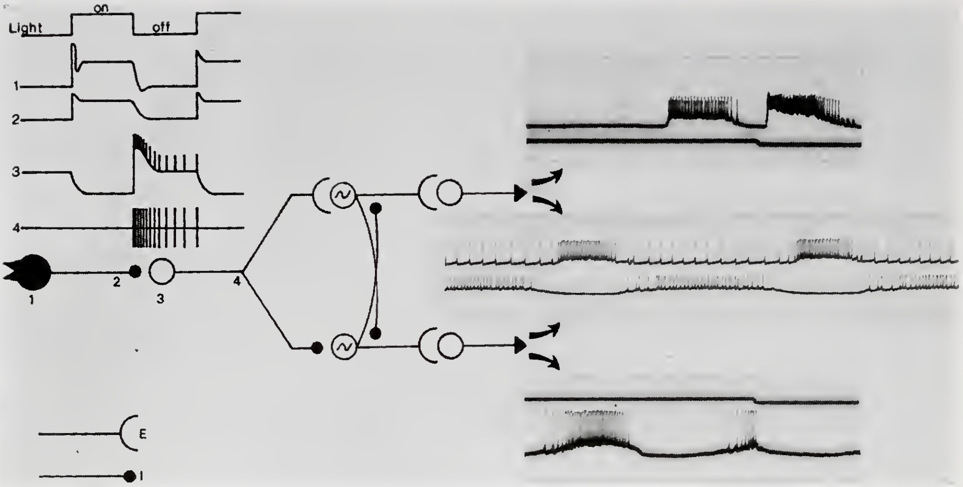


FIGURE 9. Hypothetical circuit to account for reciprocity in the output of the burster cells in *Balanus*. Upper left inset illustrates events at the numbered positions indicated on the diagram. Upper trace indicates light-on (upward deflection) and light-off (from Millecchia and Gwilliam, 1972). 1 shows photoreceptor; 2, photoreceptor axon; 3, second-order cell in supra-esophageal ganglion; 4, fibers in circumesophageal connective. Sine-wave symbol indicates an "oscillator" of unknown type. Upper and lower records illustrate effect of "light-off" (downward deflection of second beam) on LEB and MIB respectively. Middle traces illustrate normal bursting reciprocity. E. represents an excitatory synapse; I an inhibitory synapse.

report is an initial survey and is intended to be primarily descriptive of the neurons, their locations in the ganglion, and some of the properties that will permit characterization of the units. The rigorous demonstration of particular units controlling particular muscles has begun, and the general temporal relationship between the bursters and actions of muscles in extension and retraction has been established.

While the presence of a number of types of cells is recognized, interest has centered on those cells designated as bursters. This is not to imply that other units lack patterned activity, but the same kind of link to behavior as is seen in the bursters has not been established.

If one examines the output of the burster cells, it is not necessary to invoke peripheral inhibition to account for the normal extension-retraction activity. It can be seen from the records that the two types of bursters are reciprocal, that one is inhibited and the other is excited by the shadow. This kind of neuronal activity can account for much of the behavior in the intact animal given the assumption, supported by evidence, that these bursters are indeed motor neurons driving the appropriate muscles.

The output recorded from the bursters is quite variable. Much of the variability in amplitude of electrical events can be explained by invoking electrode tip position in relation to a distant spike generating site and synaptic region. The potential changes seen in the bursters consist of spikes, events best described as IPSPs and EPSPs, and slow oscillatory potential changes. Post-synaptic events without spikes or slow oscillatory potentials are commonly seen (Fig. 7C, D), and slow oscillatory events without obvious post-synaptic potentials and/or spikes are also sometimes seen. The spikes and post-synaptic potentials have an obvious explanation, but the slow potentials are not as readily explained. The variety of forms the slow changes display suggests they may be sensitive to electrode tip position and are the product of synaptic bombardment. The oscillations cannot be suppressed by passing hyperpolarizing current, but the spikes can. Excess magnesium does suppress the slow oscillations, the post-synaptic potentials, and in many cases the spikes as well. Such cells are still capable of spiking, as can be demonstrated by depolarizing the somata through the recording electrode. All of this leads to the conclusion that the patterned output of these cells is the result of the activity of presynaptic units.

The reciprocity of the patterns of the two types of bursters is also evidently due to presynaptic units. In no case have I been able to demonstrate any direct coupling between any of the bursters as tested by observing the influence of the activity of one cell upon another. One rather striking feature of all multiple site recordings, however, is that in any given preparation the periodicity of bursting patterns recorded from up to four sites is the same at any given time (Gwilliam and Bradbury, 1971, Fig. 3). If arhythmicity or silence occurs (as it does spontaneously quite frequently), it happens throughout the system. These observations suggest that one or a few units or a single "system" paces all of the output. Reciprocity in the output, however, is usually taken to imply a minimum of two units, each with inhibitory connections to the other. A recent model postulates a commonly observed phenomenon, Post Inhibitory Rebound, as the mechanism underlying alternating patterns (Perkel and Mulloney, 1974), but other theoretical possibilities exist (*e.g.*, Dagan, Vernon and Hoyle, 1975).

Such a simple system would account for the output of the bursters except for the appearance of IPSPs during the burst. It is not clear that these IPSPs are phasic and occur only on the falling phase (see Fig. 7F) but if they are, a possible source is direct reciprocal inhibition from the oscillator.

A diagram of a circuit that could account for the burster output and its interruption by a shadow is shown as Figure 9. It ignores all other inputs, which must be numerous, that would account for other modulations of output. The observation that stimulation of the cirral nerves and the great splanchnic nerve will cause the same reciprocal inhibition-excitation in the burster as a shadow is clear evidence that other inputs to the system are operative (see Fig. 3F), although it is not clear whether they act on the oscillators, on other presynaptic units, or on the bursters directly.

The diagram is not meant to imply that the "oscillators" are single units, but only that there is a part of the system that produces reciprocal bursts, that it is physically separate from the cells designated in this paper as bursters, and that it is spontaneous. Nor does this model speak to the mechanism of burst generation. Non-endogenous burster models developed to explain pattern generation in the lobster stomato-gastric ganglion (Warshaw and Hartline, 1974; Mulloney, Budelli and Perkel, 1975) may well be applicable, but there is no hard evidence that permits a choice to be made.

It has been possible in this study to record from reciprocating pairs of neurons, and such recordings do not show any signs of direct inhibitory coupling. Recordings from in-phase pairs also fail to reveal any direct coupling. Such negative evidence, however, cannot be regarded as conclusive (Selverston and Mulloney, 1974). Coupling may exist within functional subsets (*e.g.*, between cirral flexor and its antagonist which may be one or more of the muscles acting on the hydrostatic skeleton at some distant point), and would only be seen as a lucky accident of recording. Recording from in-phase pairs that are located physically close together is a more reliable test of coupling and such recordings demonstrate a common antecedent neuron as the mechanism of synchronization.

The cells described here may be compared to the bursty cells seen in the lobster stomatogastric ganglion (Mulloney and Selverston, 1974a, b; Selverston and Mulloney, 1974) and to the neurons controlling ventillation in the locust abdomen (Burrows, 1974). Some of the cells in the barnacle produce bursts of activity that are remarkably similar to the locust motoneurons both in burst fine structure and timing. The methods of activation also seem to be similar, *i.e.*, the bursters are synaptically driven and/or modulated by antecedent neurons that account for the patterning. While the patterns in these units may resemble those seen in the stomatogastric ganglion, there is no evidence of the extensive electrotonic coupling or indeed any direct synaptic connections as have been established in those motor neurons.

Another question that must be addressed is the numbers of cells referred to as bursters. If they are indeed motor neurons then there should be some correspondence between cell numbers and motor axons. That question has not been seriously considered in this work, partly because details of motor supply to the muscles are not very well known in barnacles. Counts of muscle fibers in a pair of rami from a single cirrus show that the rami together contain a total of about 70 muscle fibers. In addition, there are several fibers in the base, so a reasonable guess would be about 100 muscle fibers per cirrus. Some intracellular records from muscles show dual innervation (*i.e.*, a "small" and "large" junction potential in a single fiber as well as multiple muscle fiber activated by a single motor neuron). The degree of flexion in a cirrus achieved by driving one cirral motor neuron (LEB) would suggest each neuron innervates more than one muscle fiber, but these observations are still in the preliminary stage, and the objectivization of muscle contraction in the cirri is proving technically difficult. The use of a transducer tube (RCA 5734) will probably make this possible and permit direct studies of tension as related to motor cell activity.

The work with *Balanus hameri* was carried out at the Marine Sciences Laboratory, Menai Bridge, Anglesey, North Wales, U. K. I wish to thank Professor D. J. Crisp for permission to work at the laboratory. I especially wish to thank Dr. Derek A. Dorsett, his associates and students, who provided me with equipment, invaluable assistance, and an atmosphere conducive to work. Diolch yn fawr!

It is also a pleasure to acknowledge my gratitude to Ms. Andrea Frost for her able assistance over the past three years.

SUMMARY

The isolated barnacle central nervous system has been used to study neurons related to the shadow reflex and rhythmical behavior. The function of certain of the neurons has been ascertained by exploiting a preparation that included three pairs of cirri and the scutal adductor muscle which permitted simultaneous neuron-

muscle fiber intracellular recording. The inclusion of the median photoreceptor in all of the preparations provided a means of assaying the effect of shadows on the system.

The absence of peripheral structures in the isolated preparation coupled with the observation that rhythmical bursting patterns will persist for hours is strong evidence for one or more spontaneous "oscillators" in the CNS that are capable of driving the basic fishing, pumping behavioral repertoire of the barnacle.

The principle finding reported is that there are two classes of bursters, identifiable by location, bursting pattern, and response to "light-off" that exhibit reciprocity. Evidence is presented to link the laterally located bursters (excited at "light-off") to withdrawal, the mid-line bursters (inhibited at "light-off") to extension, in the intact animal.

Evidence the bursters are driven and/or modulated by synaptic input is provided by observations on nonspiking bursters, by passing hyper- and depolarizing currents, and by treating the ganglion with high Mg^{2+}, low CA^{2+} artificial sea water. In some of the bursters, synchronization is accomplished by a common antecedent interneuron.

Simultaneous neuron-muscle recordings and locating axons of impaled neurons in peripheral nerves has established some of the bursters as motor neurons.

LITERATURE CITED

Barnes, H., and E. S. Reese, 1960. The behavior of the stalked intertidal barnacle *Pollicipes polymerus* J. B. Sowerby, with special reference to its ecology and distribution. *J. Animal Ecol.*, **29**: 169–185.

Batham, E. J., 1944. *Pollicipes spinosus* Quoy and Gaimard, I: Notes on biology and anatomy of adult barnacle. *Trans. Roy. Soc. N. Z.*, **74**: 359–374.

Blatchford, J. G., 1970. Possible circulatory mechanism in an operculate cirripede. *Comp. Biochem. Physiol.*, **34**: 911–915.

Bullock, T. H., and G. A. Horridge, 1965. *Structure and function of the nervous systems of invertebrates, Volumes I and II.* W. H. Freeman, San Francisco and London, 1719 pp.

Burrows, M., 1974. Modes of activation of motoneurons controlling ventilatory movements of the locust abdomen. *Phil. Trans. Roy. Soc. London Ser. B*, **169**: 29–48.

Cornwall, I. E., 1953. The central nervous system of barnacles (Cirripedia). *J. Fish. Res. Board Can.*, **10**: 76–84.

Crisp, D. J., and A. J. Southward, 1961. Different types of cirral activity of barnacles. *Phil. Trans. Roy. Soc. London Ser. B*, **243**: 271–308.

Dagan, D., L. H. Vernon, and G. Hoyle, 1975. Neuromimes: Self-exciting alternate firing pattern models. *Science*, **188**: 1035–1036.

Gainer, H., 1972. Electrophysiological behavior of an endogenously active neurosecretory cell. *Brain Res.*, **39**: 403–418.

Gutmann, W. F., 1960. Functionell Morphologie von *Balanus balanoides*. *Abh. Senckenberg. Naturforsch. Ges.*, **500**: 1–43.

Gwilliam, G. F., 1963. The mechanism of the shadow reflex in Cirripedia. I. Electrical activity in the supraesophageal ganglion and ocellar nerve. *Biol. Bull.*, **125**: 470–485.

Gwilliam, G. F., 1965. The mechanisms of the shadow reflex in Cirripedia. II. Photoreceptor cell response, second order responses, and motor cell output. *Biol. Bull.*, **129**: 244–256.

Gwilliam, G. F., 1968. Spontaneous rhythmical activity in the isolated barnacle central nervous system. *Amer. Zool.*, **8**: 779.

Gwilliam, G. F., 1973. Reciprocal bursting patterns in barnacle central neurons. *Amer. Zool.*, **13**: 1303.

Gwilliam, G. F., and J. C. Bradbury, 1971. Activity patterns in the isolated central nervous system of the barnacle and their relation to behavior. *Biol. Bull.*, **141**: 502–513.

Gwilliam, G. F., and R. J. Millecchia, 1975. Barnacle photoreceptors: Their physiology and role in the control of behavior. *Prog. Neurobiol.*, **4**: 211–239.

Hoyle, G., 1964. Exploration of the mechanisms underlying behavior in insects. Pages 346–376 in R. F. Reiss, Ed., *Neural theory and modeling.* Stanford University Press, Stanford, California.

Hoyle, G., and T. Smyth, Jr., 1963. Neuromuscular physiology of giant muscle fibers of a barnacle, *Balanus nubilis* Darwin. *Comp. Biochem. Physiol.*, **10**: 291–314.

Kandel, E. R., E. R. Frazier, and R. E. Coggeshall, 1967. Opposite synaptic action mediated by different branches of an identifiable interneuron in *Aplysia.* *Science*, **155**: 346–348.

Lantz, R. C., and R. J. Millecchia, 1975. Identification of Gamma-Aminobutyric Acid as a neurotransmitter in the barnacle shadow reflex pathway. Abstract 884, *Neurosciences Abstracts*, **1**: 567.

Mendelsohn, M., 1971. Oscillator neurons in crustacean ganglia. *Science*, **171**: 1170–1173.

Millecchia, R., and G. F. Gwilliam, 1972. Photoreception in a barnacle: Electrophysiology of the shadow reflex pathway in *Balanus cariosus.* *Science*, **177**: 438–441.

Mulloney, B., and A. I. Selverston, 1974a. Organization of the stomatogastric ganglion of the spiny lobster. I. Neurons driving the lateral teeth. *J. Comp. Physiol.*, **91**: 1–32.

Mulloney, B., and A. I. Selverston, 1974b. Organization of the stomatogastric ganglion of the spiny lobster. III. Coordination of the two subsets of the gastric system. *J. Comp. Physiol.*, **91**: 53–78.

Mulloney, B., R. Budelli, and D. H. Perkel, 1975. Electrical synapses and the generation of complex motor patterns. Abstract 1016, *Neuroscience Abstracts*, **1**: 656.

Pearson, K. G., and C. R. Fourtner, 1975. Nonspiking interneurons in walking system of the cockroach. *J. Neurophysiol.*, **38**: 33–52.

Perkel, D. H., and B. Mulloney, 1974. Motor pattern production in reciprocally inhibitory neurons exhibiting postinhibitory rebound. *Science*, **185**: 181–183.

Rubin, R., 1970. The role of calcium in the release of neurotransmitter substances and hormones. *Pharmacol. Rev.*, **22**: 389–428.

Selverston, A. I., and B. Mulloney, 1974. Organization of the stomato-gastric ganglion of the spiny lobster. II. Neurons driving the medial tooth. *J. Comp. Physiol.*, **91**: 33–51.

Sommer, H-H., 1972. Endogene und exogene Periodik in der Aktivität eines niederen Krebes (*Balanus balanus* L.). *Z. Vergl. Physiol.*, **76**: 177–192.

Southward, A. J., 1955a. On the behavior of barnacles. I. The relation of cirral and other activities to temperature. *J. Mar. Biol. Ass. U. K.*, **34**: 403–422.

Southward, A. J., 1955b. On the behavior of barnacles. II. The influence of tide-level and habitat on cirral activity. *J. Mar. Biol. Ass. U. K.*, **34**: 423–433.

Southward, A. J., 1957. On the behavior of barnacles. III. Further observations on the influence of témperature and age on cirral activity. *J. Mar. Biol. Ass. U. K.*, **36**: 323–334.

Southward, A. J., 1962. On the behavior of barnacles. IV. The influence of temperature on cirral activity and survival of some warm water species. *J. Mar. Biol. Ass. U. K.*, **42**: 163–177.

Southward, A. J., 1964. The relationship between temperature and rhythmical cirral activity in some Cirripedia considered in connection with their geographical distribution. *Helgolaender. Wiss. Meeresunters.*, **10**: 391–403.

Southward, A. J., and D. J. Crisp, 1965. Activity rhythms of barnacles in relation to respiration and feeding. *J. Mar. Biol. Ass. U. K.*, **45**: 161–185.

Strumwasser, F., 1965. The demonstration and manipulation of a circadian rhythm in a single neuron. Pages 442–462 in J. Aschoff, Ed., *Circadian clocks.* North-Holland Publishing Co., Amsterdam.

Warshaw, H. S., and D. K. Hartline, 1974. A nework model of the pyloric rhythm on the lobster stomatogastric ganglion. Abstract 718, *Society for Neuroscience*, 4th annual meeting.

Willows, A. O. D., 1967. Behavioral acts elicited by stimulation of single identifiable brain cells. *Science*, **157**: 570–574.

Reference: *Biol. Bull.* **151**: 161–181. (August, 1976)

THE CHEMICAL COMPOSITION AND MECHANICAL PROPERTIES OF THE HINGE LIGAMENT IN BIVALVE MOLLUSCS[1]

GEORGE A. KAHLER,[2] FRANK M. FISHER, JR., AND RONALD L. SASS

Biology Department, Rice University, Houston, Texas 77001 and The Marine Biological Laboratory, Woods Hole, Massachusetts 02543

Members of the molluscan class Bivalvia have the shell as two calcareous valves which articulate dorsally in the cardinal region to form a hinge. In the area of the hinge is a secreted proteinaceous structure, the ligament, which is common in most members of the class. The ligament acts in opposition to the adductor musculature and functions in opening the two portions of the shell. The form of the ligament varies throughout the Bivalvia; however, in this study only two fundamental mechanical arrangements are considered. When the ligament is dorsal to the pivotal axis, the ligament is subject to tensile stress upon contraction of the adductor musculature. Alternatively, the ligament is positioned between two functionally different areas of the hinge. The part of the ligament ventral to the pivotal axis undergoes compression when the adductor musculature contracts, and it is this part of the system which is responsible for the mechanical characteristics of this type of ligament (Trueman, 1949, 1953; Alexander, 1966). The portion of the ligament dorsal to the pivotal axis is a rigid structure which maintains the juxtaposition of the valves. This latter condition is thought to be the most recent (Dall, 1895), and it is the most common form in extant bivalves. In such forms as *Mytilus edulis*, however, there may be considerable extension and contraction of the outer ligament during movement of the valves (Trueman, 1951).

Contraction of the adductor musculature stores energy in the ligament and relaxation of this musculature results in the release of the stored energy and opening of the valves. Some energy is probably lost as heat; however, the major increment is used in opening the shell.

The animals chosen for this study represent three different modes of life among bivalve molluscs. The swimming forms are represented by *Aequipecten irradians* and *Placopecten magellanicus.* Both species swim by rapidly opening and closing the valves. During swimming the rapid closing of the valves by the large, single adductor muscle, expulses water dorsally with a resultant ventrally directed movement of the animal. The "recovery stroke" or opening of the valves is executed by the inner ligament which is compressed during adduction. These animals are capable of about three opening and closing cycles per second (Alexander, 1966). The inner ligament is reminiscent of a small piece of rubber; it is black, apparently amorphous and without growth lamellae (Fig. 1a). This ligament is mounted on an extension of the shell, the chrondrophore. The outer ligament is minute in trans-

---

[1] This research sponsored in part by grants-in-aid from The Moody Foundation (70–115), and the U. S. Public Health Service (AM 18582).

[2] Current address: Biology Department, Cape Cod Community College, Hyannis, Massachusetts.

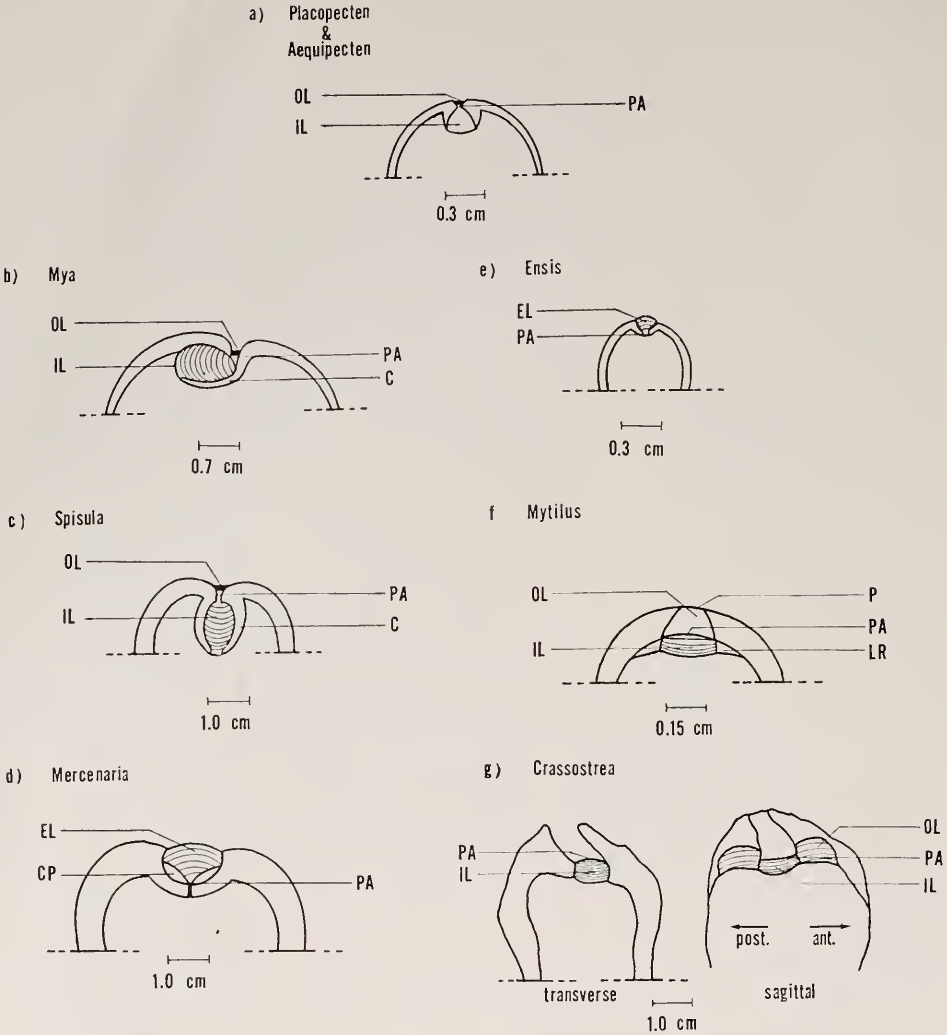

a) Placopecten & Aequipecten
OL
IL
PA
0.3 cm
b) Mya
OL
IL
PA
C
0.7 cm
e) Ensis
EL
PA
0.3 cm
c) Spisula
OL
IL
PA
C
1.0 cm
f Mytilus
OL
IL
P
PA
LR
0.15 cm
d) Mercenaria
EL
CP
PA
1.0 cm
g) Crassostrea
PA
IL
OL
PA
IL
post.
ant.
transverse
sagittal
1.0 cm

FIGURE 1. Schematic versions of transverse sections through the major ligaments of bivalve molluscs. The growth lamellae visible on the ligaments are indicated where present by repeating lines. These diagrams serve to demonstrate only mechanically functional properties and readers should refer to Owen, Trueman and Yonge (1953) for developmental and morphological details of the ligament. Abbreviations are C, chrondophore; CP, calcified attachment plate; EL, external ligament; IL, inner ligament; LR, ligamental ridge; OL, outer ligament; P, periostracum; and PA, pivotal axis.

verse section; however, it is greatly elongated in the anterio-posterior direction. This portion of ligament contributes little to the mechanical properties of adduction (Trueman, 1949, 1953; Alexander, 1966). The valves of the Pectinidae have no interlocking teeth in the cardinal region of the shell. The elongated outer ligament serves to maintain the juxtaposition of the valves at the point of articulation. *Mya* *arenaria, Spisula solidissima, Mercenaria mercenaria,* and *Ensis directus* are representative of the burrowing bivalves. In these species the ligament is not always capable of opening the valves against the force exerted by the mud or sand in which they live; hence, secondary mechanisms may be operative (Truman, 1954). The ligament of *S. solidissima* (Fig. 1c) may, however, be of sufficient strength to open the shell without aid (Russell-Hunter and Grant, 1962). External ligaments which are thought to be "primitive" (Dall, 1895) are found in *M. mercenaria* and *E. directus* (Fig. 1d and e). Such ligaments are partially under tensile stress when the valves are closed. The other two burrowers display the more "advanced" ligament in which the main body of the ligament has shifted to a position ventral to the pivotal axis of the valves and is thus compressed by the action of the adductors. The ligaments of both *M. arenaria* (Fig. 1b) and *S. solidissima* (Fig. 1c) are mounted on chondrophores. A long chondrophore provides the ligament with a lever to the pivotal axis and, therefore, a mechanical advantage in opening the valves.

Many species of bivalves are sessile or semi-sessile. *Mytilus edulis* and *Crassostrea virginica* (Fig. 1f and g) are characteristic of this mode of life. The resistance to opening the valves encountered by burrowing species is not a problem for attached forms, unless they are living in extremely crowded communities. The gape of the valves of attached species tends to be slight and the rate of opening slow; this is in marked contrast to the Pectinidae.

Growth lamellae are often present in the ligament tissue and represent periods of varying growth potential. The dorsal area of the mantle, the isthmus, is responsible for secretion of the inner ligament. Consequently, the growth lamellae are oriented parallel to the surface of the underlying mantle. The outer layers of the ligament are secreted by the outer lobe of mantle margin (Owen, Trueman and Yonge, 1953).

Most of the publications of the last century which discussed the bivalve ligament were concerned with the phylogenetic relationships (Dall, 1889; Jackson, 1890, 1891); however, Dall (1895) did propose the name "resilium" for the internal ligament in an attempt to indicate its function. Dall (1895) described the function of the primitive ligament as having the essential nature of a C-spring.

The investigations of Trueman (1942, 1949, 1950a, 1950b, 1951, 1953, 1954, 1964, 1966) represent the first and, with few exceptions until this report, the only attempt to approach the study of bivalve ligaments from the aspect of functional morphology. Trueman (1942), in describing the ligament of *Tellina tenuis*, noted birefringence in several parts of the ligament but was unable to deduce its nature. In 1949, Trueman speculated that the birefringence might be due to some substance of lipoid nature. The outermost ligamental layers of *Mytilus edulis* were also reported (Trueman, 1950a) to display birefringence. The staining properties of the ligament can be used to demonstrate that the outer and inner layers of ligament from various bivalves are similar. With Mallory's triple stain the outer layers stain red and the inner layers blue (Trueman, 1951). The ligamental layers and the inner and outer calcareous layers of the shell are derived from the same structure, the mantle, and comparison of the growth lines of the ligament and the corresponding lines in the valves is possible. Both layers of the ligament are considered to be conchiolin (the organic phase of shell) as the ligamental layers represent local modifications of shell layers (Owen *et al.*, 1953; Trueman, 1964). The conchiolin of *Anodonta* ligament is heavily tanned by orthoquinones in the outer layer, but only slight tanning occurs in the inner layer (Trueman, 1950b). Heavily tanned outer ligaments seem to be of general occurrence. This sclerotization of the outer ligament with chemical bonding of adjacent polypeptide chains enhances the mechanical ability of the ligament to withstand tensile stress (Trueman, 1964). Recently Andersen (1967) has isolated 3,3′-methylene-bistyrosine from whole ligaments of *Mytilus edulis* and the inner ligaments of *Spisula solidissima* and *Pecten maximus*. This compound represents, according to Anderson (1967), a unique method of protein tanning. The two most common elastic proteins are cross-linked with di-tyrosine and tri-tyrosine (resilin from insect cuticle), and desmosine and isodesmosine (elastin, the vertebrate elastic protein). Beedham (1958) conducted an amino acid analysis on the ligament of *Anodonta*, but unfortunately he used visual assessment of areas on paper chromatographs to assay the amount of a particular amino acid in a ligament hydrolyzate. He suggested the relatively high proline content and very low percentage of phenolic amino acids indicated a composition similar to collagen; but the relatively low proportion of glycine, little or no hydroxyproline, and appreciable quantities of methionine were quite unlike collagen. The noncollagenous nature of the ligament was reaffirmed by Hare (1963) who reported no hydroxylysine or hydroxyproline in the ligament of *Mytilus californianus*. However, Hare (1963) found 391 glycine residues per thousand in the outer ligament and 197 in the inner ligament. Both the ligament of *Aequipecten irradians* and *Placopecten magellanicus* were reported to have over 600 glycine residues per thousand (Kelly and Rice, 1967).

Kelly and Rice (1967) further proposed the name "abductin" for the protein of the inner ligament of the "scallop" in order to indicate its function. "Native type" collagen fibrils with an axial repeat generally less than 600 Å have been noted as a minor component of mollusc shell matrix protein (Travis, Francois, Bonar, and Glimcher, 1967). However, unlike ligament, hydroxyproline and hydroxylysine have been reported from mollusc shell matrix protein (Piez, 1961). Only Kelly and Rice (1967) have reported X-ray diffraction patterns for ligament, and they indicate no distinct pattern from the pectens. Galtsoff (1964) published electron micrographs of *Crassostrea virginica* inner ligament (resilum) fixed with 1% osmic acid. A section across the plane of the growth lamellae revealed a honeycomb appearance with holes about 500 Å in diameter. Another section, apparently at right angles to the holes, revealed fibrils varying in diameter from 370 to 500 Å. It is interesting that Travis *et al.* (1967) reported the presence of holes or compartments in sheets of decalcified shell matrix protein. Furthermore, they reported that these holes contained the inorganic crystals and this arrangement was common to all mineralized tissues, both invertebrate and vertebrate thus far studied. Bevelander and Nakahara (1969) maintained that the conchiolin of *Mytilus edulis* and *Pinctada radiata* is homogeneous and not composed of a fibrillar structure.

The organic phase of ligament is often associated with an inorganic phase, $CaCO_3$. Very little research has been directed toward this aspect of ligament morphology. Trueman (1949) reported that treatment with dilute hydrochloric acid indicated the presence of calcareous material in the periphery of the inner ligament of *Tellina tenuis*. Trueman (1964) stated that, in general, outer ligament contains no calcium carbonate and inner ligament typically consists of relatively little tanned protein, and calcium carbonate. Stenzel (1962) noted that aragonite was the crystalline phase of calcium carbonate in the resilium of *Crassostrea virginica*. Hare (1963) reported aragonite in *Mytilus californianus* ligament, and Bevelander and Nakahara (1969), in a paper concerned with the synthesis of ligament, published electron micrographs of aragonite crystals in the ligament of *Mytilus edulis*.

Trueman (1951) made the first measurements of the physical characteristics of the ligament. He estimated the mass which the valves of *Ostrea edulis* were capable of moving under the influence of the ligament and compared this with the surface area of the valves to determine the relative capability of valve abduction for several bivalve species. The first hysteresis loops for bivalve ligament were published by Trueman (1953) for several species of bivalves. He concluded from this type of data that the outer ligament of *Pecten maximus* probably behaves as a fairly rigid hinge structure. Trueman (1953) also used the percentage difference of the closing and opening moments (very nearly the torque generated by the ligament at closing and the torque generated at opening, respectively) as a measure of the ligament. The difference is about 4% for *Pecten* and about 10 to 20% for other bivalves. Trueman (1953) showed a relatively low internal resistance in *Pecten*, suggesting a more efficient mechanism for the frequent opening and closing of the valves. The unusual efficiency of the ligament of these bivalves is undoubtedly related to the swimming mode of life. Trueman (1953) also stated that the area enclosed by a hysteresis loop is a measure of ligament efficiency, but he did not make this measurement. He did observe, by inspection, that *Pecten* and *Chalmys* ligaments generated hysteresis loops with markedly less enclosed area than most bivalves.

More recent studies on the mechanical properties of ligament were conducted by Russell Hunter and Grant (1962) using *Spisula solidissima*. They concluded that the ligament was the main mechanism opening the shell of this burrowing bivalve. The secondary mechanisms of abduction, mentioned above, were apparently not required in this clam. Alexander (1966) in a lengthy thermodynamic approach to the study of the inner ligament of the Pectinidae concluded that this tissue functions with rubber-like elasticity, in which changes of entropy are more important than changes of internal energy.

The ligament of the bivalve molluscs is thus an unusual structure in that it is a nonliving tissue which carries out a function normally reserved for muscle, *i.e.*, it opposes the action of a flexor muscle. It is the intent of this paper to compare physically and chemically the functional structure of the ligaments of a variety of bivalve molluscs representative of differing life styles.

MATERIALS AND METHODS

Source and maintenance of experimental animals

The molluscs used in this study, *Placopecten magellanicus* (Gmelin), *Aequipecten irradians* (Lamarck), *Ensis directus* (Conrad), *Mya arenaria* (L.), *Spisula solidissima* (Dillwyn), *Mercenaria mercenaria* (L.), and *Mytilus edulis* (L.), were obtained from the Supply Department of the Marine Biological Laboratory, Woods Hole, Massachusetts. Measurements of the mechanical properties of the ligament of these animals were made at the Marine Biological Laboratory with animals maintained in the running seawater system at 18 to 20° C. *Ensis directus* was maintained in trays of sand while the other species were placed on the bottom of the sea table in the running water.

Crassostrea virginica was obtained from commercial fishermen in Kemah, Texas, and maintained in a recirculating artificial seawater (Seven-Seas Marine Mix, Utility Chemical Company) system of 150 gallons capacity until the mechanical properties of the ligament were measured. The artificial sea water was admixed with equal portions of water from the Gulf of Mexico and the salinity adjusted to 33‰ with tap water. The temperature range of the seawater system was 17 to 20° C. No effort was made to retard natural growth of microorganisms or other sources of suspended food in the system. Calcium carbonate and chalk were added to the water to aid in the maintenance of pH and as a supply of calcium ions. The circulating water was passed through glass wool, activated charcoal, and a layer of crushed oyster shell which acted as a filter as well as aerating the system. Several times during the course of the project it was necessary to replenish the supply of animals from the New England area. Live animals were shipped by air freight from the MBL in chilled insulated containers and maintained in the seawater system at Rice University prior to experimentation.

Chemical analysis

Ligament tissue for chemical analysis was carefully dissected away from the valves, rinsed with distilled water, and ground with a mortar and pestle. The powder was dried, *in vacuo*, for 24 hours at 22° C and stored over silica gel at room temperature until preparation for analysis. No chemical change was noted during storage. Fresh ligament tissue was obtained from the animals maintained in the seawater system at Rice University and prepared immediately for analysis.

Amino acids and protein. Amino acid analysis of the ligament tissue was conducted on acid hydrolyzates of the above powder. Samples of 10 to 40 mg were hydrolyzed in 2 ml of 6 N HCl in sealed vials for 24 hours at 120° C (Campbell, 1960). The acid was removed from the hydrolyzate by low temperature vacuum distillation in a "Rotary Evapo-Mix" (Buchler Instruments Co.) and the residues were returned to a known volume in 0.1 N HCl. Since no interference was observed, no attempt was made to remove inorganic ions prior to analysis on an amino acid analyzer. Concentrations of amino acids as low as 10^{-8} M were measurable with this methodology.

A stoichiometric relationship may be assumed between the amide N and the number of asparagine and glutamine residues (Hare, 1963). The amide N was released from the ligament powder by boiling with 2 N HCl (Chibnall, Mangan and Rees, 1958) and the resultant, NH_4Cl, was assayed by the method developed by Seligson and Seligson (1951) as described by Campbell, Bonner and Lee (1968) with the following modification: prior to addition of the K_2CO_3, 0.18 ml of 60% KOH was added to each vial. The addition of this base was necessary because the NH_4Cl was in 2 N acid rather than the 0.1 N acid as described by Campbell, Bonner and Lee (1968). The addition of this base resulted in an increase in the pH to about 1.2, which compared with the pH of the 0.1 N acid used by Campbell *et al.*, and favored the release of ammonia from solution after addition of the K_2CO_3.

Standard solutions containing nitrogen in 2 N acid were made to correspond to the samples. Release of amide N from *S. solidissima* ligament in 2 N HCl at 100° C was complete within 30 minutes. The hydrolysis conditions used for all experiments to measure amide N were 1 ml of 2N HCl per 6 mg or less dried hinge ligament at 95 to 100° C at 1 atm for 1 hour. Recovery of amide N, under these conditions, averaged 87 ± 5.6% and 91 ± 5.9% (s.e.) from authentic samples of asparagine and glutamine, respectively.

Total protein was estimated from Kjeldahl nitrogen assayed by the micromethod of Lang (1958). Recovery of nitrogen from lysine averaged 90 ± 1.6% of the calculated amount in a sample of known concentration. The percentage of nitrogen for each ligament protein was calculated from the amino acid analysis. Total protein was then calculated from the formula:

$$\text{T.P.} = \frac{\text{Per cent total N of ligament (Kjeldahl)}}{\text{Per cent N for ligament protein}} \times 100$$

Carbohydrate. Total carbohydrate was determined on aliquots of the acid hydrolyzate prepared for amino acid analysis using the phenol-H_2SO_4 method of Dubois, Gilles, Hamilton, Rebers and Smith (1956). Variation in sugar contents were determined in a Klett-Summerson colorimeter using a No. 50 Klett filter (470 to 530 mμ).

Lipid. The lipid content of the ligament tissue was estimated by grinding a sample of the dried ligament to a finer powder with a mortar and pestle. This powder was extracted in 2:1 (V/V) chloroform-methanol mixture (Folch, Ascoli, Lees, Meath and LeBaron, 1951) using 5 ml per 30 to 40 mg of ligament powder. The extraction was carried out for 24 hours at 22° C in glass-stoppered test tubes placed on a rotator. After extraction the tubes were cleared by centrifugation, aliquots evaporated, and weighed for lipid content.

Inorganic measurements. Calcium content of ligament tissue was measured by atomic absorption spectrophotometry from aliquots of hydrolyzate prepared for amino acid analysis. To eliminate possible interference by various substances (such as amino acids) the method of standard additions was used (Christian and Feldman, 1970). One per cent lanthanum was included in all standards and samples to reduce flame stable complexes of calcium. All standards and samples contained 0 to 10 micrograms of calcium per ml of 1% lanthanum in 0.6 N HCl. The atomic absorption spectrometer was a "Norelco Unicam, SP90A." All instrument parameters—fuel flow, air flow, slit width, etc.—were those recommended by the manufacturer for the determination of calcium (Willis, 1960).

Crystalline phase of the calcium carbonate was determined by X-ray diffraction. Samples were ground to a fine powder and spread in a thin, even layer on one side of double surface cellulose tape. The clean side was affixed to a petrographic slide to permit attachment to the goniometer head. Intensities of diffracted beams were recorded on a standard wide angle Norelco X-ray diffractometer equipped with an automatic recording device. Nickel filtered copper K_α radiation (35 kV and 18 mA) was used throughout this procedure.

Mechanical properties of ligament

Measurement of hysteresis. Mechanical hysteresis properties of the ligament of each species of bivalve were obtained from animals in which the adductor muscle had been severed and all the tissue teased from the right valve. The experiments were conducted immediately at room temperature. The valves were never permitted to gape more than the maximum observed when the animal was alive. The apparatus used for these measurements is similar to that used by Trueman (1951) and Russell Hunter and Grant (1962). The load (L) was recorded for each angle of gape. In most cases several cycles from fully open (fully open = maximum gape observed in the living animal) to fully closed were estimated. The weight of the right valve (W) and the distance from the centroid of the shell to the center of the hinge (d) were also recorded. The torque, or moment of force about the hinge (M), can be calculated from the formula: $\mathrm{M} = d(2L + W)g$, where g is the acceleration of gravity. The constant, 2, reflects the mechanical advantage of the lever system. It has been customary to drop the gravitational factor from the expression (Trueman, 1951, 1953; Russell Hunter and Grant, 1962), which results in $\mathrm{M} = d(2L + W)$, where the units are then gram millimeters instead of dyne millimeters.

The angle of gape was calculated from the absolute gape (as measured from the metric scale) and the distance from the ventral lip to the center of the hinge. All calculations and graphs of hysteresis were completed with the aid of a Hewlett-Packard 9100B calculator equipped with an on-line plotter (H. P. 9125B).

Resilience. The term resilience as defined by Alexander (1968) is the work recovered from a material in an elastic recoil, expressed as a percentage of the work previously done in straining it. The resilience is equal to the area under the closing curve in Figure 2 expressed as a percentage of the area under the opening curve. The area in each case represents the product of force and distance, that is, work. Thus

$$\text{Resilience} = \frac{\text{Force} \times \text{Distance (recovered)}}{\text{Force} \times \text{Distance (put in)}} \times 100$$

$$= \frac{\text{Work (recovered)}}{\text{Work (put in)}} \times 100$$

A hypothetical curve for the hysteresis of bivalve ligament is shown in Figure 2. The area under the lower curve as a percentage of the area under the upper curve is used throughout as resilience.

$$\text{Resilience} = \frac{\int(\text{gram-millimeters}) \times d\theta}{\int(\text{gram-millimeters}) \times d\theta} \times 100$$

where θ is the angle of gape. The numerator is the area under the lower curve; the denominator is the area under the upper curve. The distance from the center of the shell to the center of the hinge (in millimeters) is a constant for each animal. The load on the valve (in grams) could be converted to force by multiplying by the acceleration of gravity. Since the load is in both the numerator and denominator this amounts to multiplication by unity. So the only major difference between this

TABLE I

Chemical composition of ligament tissue. Percentages are by weight based on samples of dried powder used for analysis. Standard error of the mean is n = 3, for protein; n = 2, for $CaCO_3$. Symbols are ND, not detected; IL, inner ligament; EL, external ligament.

	Aequipecten (IL)	*Placopecten* (IL)	*Spisula* (IL)	*Mercenaria* (EL)	*Ensis* (EL)	*Mya* (IL)	*Mytilus* (IL)	*Crassostrea* (IL)
% Protein	97.3	98.9	48.8	25.2	25.0	50.5	33.3	33.9
	±4.6	±2.1	±1.1	±1.1	±1.5	±1.4	±4.8	±1.5
% $CaCO_3$	1.5	2.1	63.9	86.2	86.5	60.5	64.9	92.0
	±.06	±.19	±6.2	±7.5	±9.9	±2.8	±3.8	±.12
% Carbohydrate	0.83	0.90	0.22	0.13	0.11	0.31	0.36	0.12
% Lipid	ND	ND	ND	ND	ND	ND	ND	ND
% Total	100	102	113	112	112	111	99	126
$\frac{\%\ CaCO_3}{\%\ \text{Protein}}$	0.015	0.021	1.31	3.42	3.46	1.20	1.95	2.71

definition and that of Alexander (1968) is the use of angular distance. While not strictly analogous to Alexander's definition this approach is sufficient to compare the resilience of several species. This approach was adopted in order to utilize the already accepted method of reporting bivalve ligament hysteresis (see Trueman, 1953, 1945; and Russell Hunter and Grant, 1962).

RESULTS

Protein, calcium carbonate, carbohydrate, and lipid were determined and the results are reported in Table I. Outer ligaments which do not contribute significant thrust to the opening of the valves (Trueman, 1949, 1953; Alexander, 1966) were not considered in the analysis. Carbohydrate was found to contribute less than one per cent of the ligamental tissue and no lipid was detected by the gravimetric method employed. The absence of lipid in this tissue is significant in relation to Trueman's (1949) suggestion that the birefringence he observed in the ligament of *Tellina tenuis* might be of lipoid nature. The ligament of *T. tenuis* was not investigated in this study, however, birefringence of a nonlipoid nature was noted in the ligament of *Spisula solidissima*. The ligament portions of the species investigated were essentially composed of protein and calcium carbonate in differing proportions. The ratio of calcium carbonate/protein varied from 0.015 for *Aequipecten* to 3.46 for *Ensis*.

The per cent calcium carbonate was calculated from the calcium ion concentration determined by atomic absorption spectrophotometry. The presence of calcium carbonate was verified by X-ray diffraction; however, in the case of *Aequipecten* and *Placopecten* ligament the presence of calcium carbonate could not be ascertained by this method. No inorganic reflections were observed with either rapidly scanning, wide angle, X-ray powder technique or a four hour, wide angle, transmission Laue exposure. Thus, there is no evidence that the relatively small calcium ion concentration, determined by atomic absorption, is representative of a calcium carbonate phase in the ligament of these two species. There are three possibilities; the ionic calcium is not from calcium carbonate, the calcium is amorphous calcium

TABLE II

Amino acid analysis of bivalve ligament and shell protein expressed as residues per 1000. A dash indicates below level of detection or not calculated.

	Aequipecten (IL)*	*Placopecten* (IL)*	*Spisula* (IL)	*Spisula* (OL)	*Mercenaria* (EL)	*Ensis* (EL)	*Mya* (IL)	*Mytilus* (IL)	*Crassostrea* (IL)	*Crassostrea*** shell protein (prismatic)
Cystine/2	2.4	3.7	10.3	8.3	20.9	14.7	15.2	12.5	18.4	7.3
Aspartic acid	17.1	60.2	23.2	47.4	115.0	239.3	41.4	38.6	199.7	122.0
Threonine	10.5	5.9	8.0	31.7	13.9	15.9	14.1	14.4	26.9	18.0
Serine	60.2	28.6	21.5	57.9	13.0	19.1	29.4	89.2	49.0	123.0
Proline	11.0	5.9	27.8	51.6	102.0	71.5	55.5	47.3	99.5	70.0
Glutamic acid	13.0	18.3	19.8	36.2	26.4	34.1	18.8	37.4	61.9	33.0
Glycine	683.9	678.5	628.5	481.2	289.7	295.1	532.0	410.5	156.9	337.0
Alanine	29.6	21.6	34.6	22.0	49.3	46.3	61.6	41.6	85.8	80.0
Valine	4.7	2.0	9.3	37.6	21.2	30.0	27.7	22.3	31.0	20.0
Methionine	59.6	88.7	132.0	26.1	230.2	134.5	133.3	96.6	158.9	3.5
Isoleucine	6.4	3.2	21.6	44.2	15.3	7.9	10.8	31.3	20.4	12.0
Leucine	1.9	2.3	18.7	32.3	17.6	35.1	10.1	72.2	16.3	38.0
Tyrosine	1.1	8.4	1.9	42.3	2.5	—	—	3.9	11.3	46.0
Phenylalanine	86.0	51.2	19.9	26.2	8.3	25.8	21.2	22.3	17.5	31.0
Lysine	8.6	12.7	10.3	16.2	42.9	19.7	7.5	34.0	14.1	15.0
Histidine	—	—	2	12.4	5.3	4.3	3.4	4.0	7.5	17.0
Tryptophan	—	—	—	—	—	—	—	—	—	—
Arginine	4.1	8.8	11.0	36.4	26.2	5.7	18.1	22.0	24.9	27.0
Hydroxylysine	—	—	—	—	—	—	—	—	—	—
Hydroxyproline	—	—	—	—	—	—	—	—	—	—
$\frac{\text{Asp} + \text{Glu} - \text{Amide N}}{\text{Lys} + \text{His} + \text{Arg}}$	0.18	1.57	1.24	—	1.60	6.39	1.20	0.98	3.98	—
Amide N	27.8	44.8	14.1	—	22.7	83.3	25.1	16.8	77.0	—

* These results are in essential agreement with Kelly and Rice (1967).

** Travis *et al.* (1967).

carbonate, or the X-ray analysis was not sufficiently sensitive to detect the crystalline phase.

Ligament tissue, like many structural proteins such as elastin, resilin, keratin, and collagen (old), are found in a solid phase which is relatively insoluble. The results of the amino acid analysis are shown in Table II. The outer ligaments were not considered; however, the outer ligament of *Spisula solidissima* was included for comparative purposes, as was the shell protein from *Crassostrea virginica*. The most striking feature of these analyses is the concentration of glycine found in the ligaments of the eight species of bivalves examined. Over 500 glycine residues per thousand total residues were measured in the ligament hydrolyzates of four of the eight species examined. Only *C. virginica* had fewer than 200 glycine residues per thousand, while the presence of nearly 70% glycyl residues occurred in *A. irradians* and *P. magellanicus* hinge ligament protein. Among the ligaments examined, *C. virginica* had the highest concentration of alanine (9%). A significant difference between ligament protein and many other structural proteins is the presence of the sulfur-containing amino acids, methionine and cystine/2. The presence of cystine/2 residues may be indicative of the degree of cross-linking in the ligament protein. It is obviously important that the cross-links be relatively few and widely spaced so that stretching may occur without rupture of strong bonds. Some cross-linking is necessary, however, to prevent the slipping of one chain past another (Weis-Fogh, 1961; Partridge, 1962), yet a considerable degree of cross-linking may be possible without impairing mechanical function. The external ligaments of *Mercenaria* and *Ensis* are subject to stretching during valve adduction. Covalent cross-linking would probably not be incompatible with this functional morphology. The added firmness resulting from cross-linking may, in part, account for the greater strength of the ligaments which contain the greatest amounts of cystine/2.

Proline residues commonly represent 10 to 20% of the total residues of structural proteins (Seifter and Gallop, 1966). The ligament proteins from the *Aequipecten irradians* and *Placopecten magellanicus* are the lowest in proline content of the bivalves examined. The absence of this amino acid could permit considerable hydrogen bonding and rubbery elasticity could be seriously impaired by extensive hydrogen bonding between polypeptide chains. The concentration of valine, phenylalanine, tyrosine and histidine are characteristically low in collagen (Harrington and Von Hippel, 1961) and a similar situation prevails in ligament protein. It should be noted that during acid hydrolysis the concentration of several amino acids may be altered. Tryptophan is completely destroyed while serine, threonine, lysine, arginine, as well as histidine may be partially degraded. The amide nitrogen

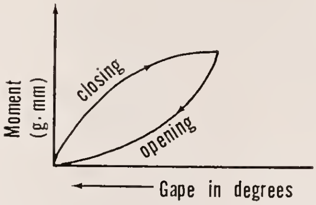


FIGURE 2. Typical mechanical hysteresis.

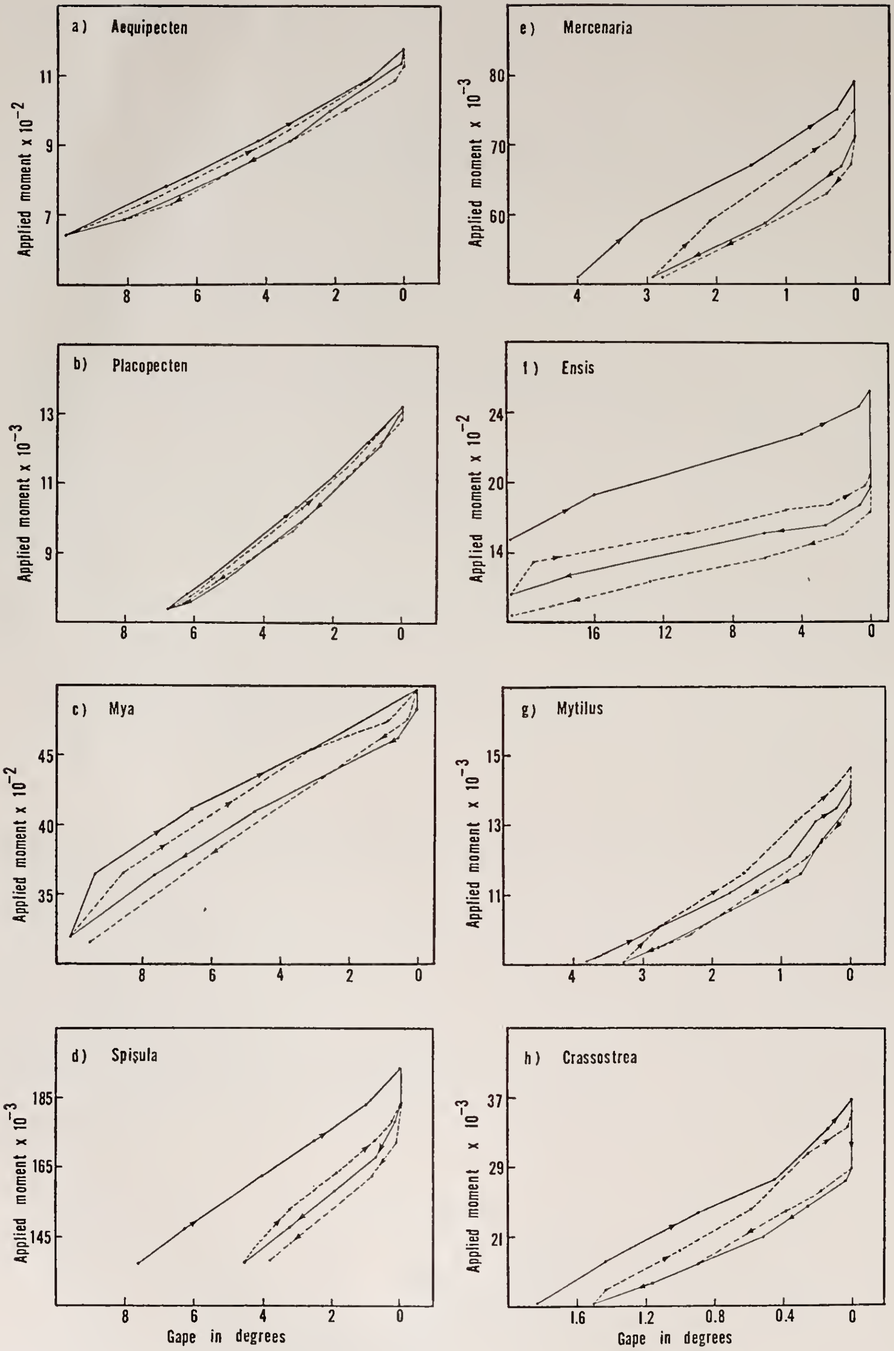


FIGURE 3. Hysteresis curves generated by the hinge ligaments from eight species of bivalve molluscs. The applied moment in g/mm is represented on the ordinate. The angle of gape

of glutamine and asparagine is hydrolyzed resulting in an apparent increase of glutamic and aspartic acids, respectively.

Both elastin and resilin are plasticized with about 50 to 60% water, and become rigid and glasslike when dried (Seifter and Gallop, 1966). Hydrated inner hinge ligament powder from *Spisula solidissima* achieved constant weight when dried *in vacuo* over $CaCl_2$ for 24 hours; the weight loss was 11.95 ± 0.44%. Drying at 98° C and ambient pressure resulted in a further loss of 2.6 ± 0.1% during the first 24 hours. No significant losses in weight were noted after 48 and 72 hours at 98° C. The ligaments of all eight species became quite hard and brittle upon drying at room temperature, yet the characteristic resilience was restored upon rehydration. Glycerol can plasticize resilin and cause it to swell (Seifter and Gallop, 1966); however, the ligament of *S. solidissima* shrinks markedly and loses its resilience when exposed to glycerol at room temperature for several days. Further, *Spisula* inner ligament was not affected by 0.5 N HCl or NaOH at room temperature, and underwent no visible changes when heated to 100° C in water for 5 to 6 hours, characteristics quite different from collagen.

Ashing *S. solidissima* inner ligament for 20 hours at 450° C resulted in recovery of 56.7 ± 0.4% of the weight of the dry powder. Atomic absorption analysis indicated 63.9 ± 6.2% calcium carbonate in the inner hinge ligament (Table I). X-ray diffraction patterns of the ash, by the powder technique, revealed strong aragonite reflections and, occasionally, weak calcite reflections. Calcite reflections never occurred in the diffraction patterns of fresh ligament; therefore, some aragonite may have shifted phase to the more stable calcite configuration during the ashing procedure. The inner hinge ligaments of *Aequipecten irradians* and *Placopecten magellanicus* presented no calcium reflections in the diffraction pattern; however, the remaining six species in this study all contained aragonite as the calcium carbonate phase in that structure.

The mechanical hysteresis of the ligaments of all eight species were examined and representative curves are shown in Figure 3. *Aequipecten irradians* and *Placopecten magellanicus* have the narrowest loops which indicate resilient ligaments while the widest loops, the least resilient ligaments, occur with *Ensis directus*. The curves indicated by broken lines are the second cycle of loading and unloading. The second curve is usually displaced to the right of the first curve. Thus, any particular moment applied to the valves usually results in a greater closure in the second load-unload cycle. Several of the curves do not form closed loops (*e.g.*, *Spisula*). In this situation the valves have failed to achieve the initial gape under the initial load conditions; however, it was observed that the valves of *Spisula* do reset in about 20 minutes.

The resilience of the ligament of each species was calculated from the hysteresis curves and the results are reported in Table III. The ligament resilience of the swimmers, *A. irradians* and *P. magellanicus*, exceeds the other species. The swimming life style required the valves to open and close rapidly; hence, a very resilient ligament is clearly advantageous to this mode of existence. Trueman (1953) reached a similar conclusion about the ligaments of *Pecten* and *Chalmys*.

---

of the valves is presented as degrees on the abscissa. The first load-unload curve is represented by the solid line; the second, by the hatched line. Arrows indicate the direction in which the right valve was moving.

Table III

Resilience of ligaments, calculated from the areas under the hysteresis curves. n = Number of hysteresis loops for which the mean and standard error were calculated. No more than two loops from any one specimen were used in the calculation.

Species	Mean resilience ± s.e.	*n*
Aequipecten	96.15 ± 0.25	12
Placopecten	96.59 ± 0.36	13
Mya	92.33 ± 0.75	9
Spisula	93.56 ± 0.69	11
Mercenaria	91.93 ± 0.68	12
Ensis	81.81 ± 2.15	8
Mytilus	93.14 ± 1.31	10
Crassostrea	83.94 ± 1.06	11

It is striking that the burrowers (except *Ensis*) have very resilient ligaments. The burrowing life style required that the valves open against the force of a sandy or muddy substrate (Trueman, 1954) which would require a slow, powerful abduction during burrowing (Russell Hunter and Grant, 1962). Trueman (1966) found that even in burrowers which rely heavily on secondary valve abduction mechanisms there is a stage during burrowing which is ligament-dependent and apparently very rapid (Trueman, 1966, stage 5). Furthermore, bivalves are known to clear the mantle of unwanted foreign matter (pseudofaeces) by occasional, rapid opening and closing of the valves.

The opening moment is that value of applied moment which is recorded as the valves just begin to gape during the unloading cycle. Russell Hunter and Grant (1962) found the opening moment of *Spisula solidissima* to be nearly constant for successive cycles of loading and unloading. Opening moments of repeated loading and unloading cycles are not constant in the eight species of bivalves examined in this study. However, the opening moment tends to be the most nearly constant value from any set of cycles; therefore, the opening moment expressed per gram of shell is taken as an indication of the absolute strength of the abduction system (Fig. 4).

Discussion

The principal feature of the molluscan hinge ligament is the high glycine content in the organic phase (Table II). Collagen is characterized by a high glycine content and the presence of 30 to 50% glycyl residues is almost thematic in structural proteins (Seifter and Gallop, 1966). The absence of hydroxyproline and hydroxylysine, the acid residues diagnostic of collagen, alludes to the noncollagenous nature of the bivalve ligament. Alanine, commonly present from 12 to 40% in structural proteins (Seifter and Gallop, 1966), is present in relatively low concentrations in the ligament hydrolyzates. Also significant of the ligament protein is the presence of sulfur containing amino acids. Both methionine and cystine/2 are characteristically very low or absent in collagen (Harrington and Von Hippel, 1961), and are similarly low or absent in elastin and resilin (Seifter and Gallop, 1966). Elastin and resilin are thought to exhibit rubbery elasticity (Weis-Fogh, 1961).

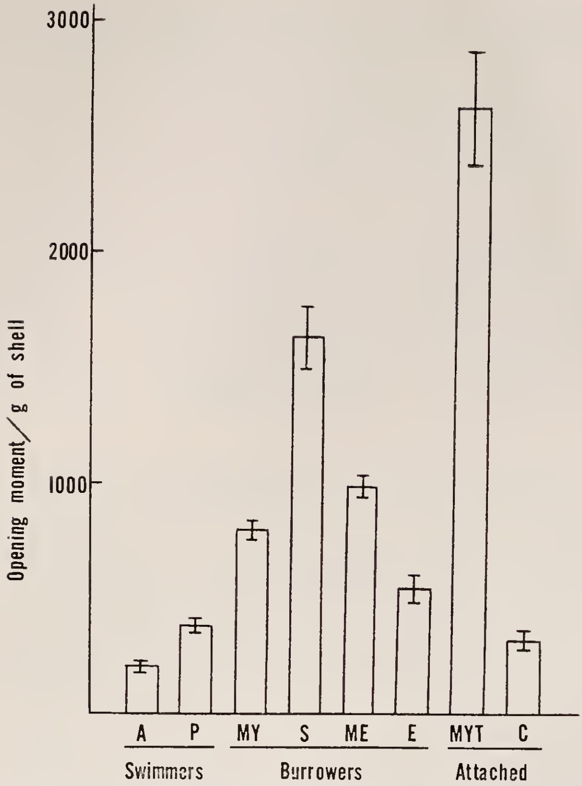


FIGURE 4. Relative strength of the abduction system in some bivalve molluscs. Abbreviations are A, *Aequipectin*; P, *Placopectin*; MY, *Mya*; S, *Spisula*; ME, *Mercenaria*; E, *Ensis*; MYT, *Mytilus*; and C, *Crassostrea*.

About 50 to 60 cystine/2 residues per thousand are found in keratin and are thought to be involved in the cross-linking of protein chains (Seifter and Gallop, 1966).

The pattern suggested by the amino acid analysis of the bivalve ligament is one of large nonpolar regions of protein (Table II). The peptide chains, unlike those of most other structural proteins, except the low glycine in keratin, may be cross-linked with disulfide bonds. It has also been reported that the ligament protein is cross-linked with 3,3′-methylene-bistyrosine (Andersen, 1967). Cross-linking may be associated with the compressible nature of the inner ligament. The presence of extensive amounts of cross-linking in elastin, and perhaps the other structural proteins, would be incompatible with the degree of stretching required of these proteins. Added stiffness, while not compatible with long tensile deformation, would add to the ligament's ability to abduct the valves.

Hydrolyzates from *Aequipecten irradians* and *Placopecten magellanicus*, the two swimming bivalves, tend to be unique with respect to the other species. They contain the greatest concentration of glycine residues, and the least amount of cystine/2 and proline (Table II). Thus, they contain the largest amounts of nonpolar amino acids, probably with very few cross-linkages or hydrogen bonds

Table IV

Correlation coefficients for sets of structural and physical parameters.

			Correlation coefficient
$CaCO_3$/protein	*vs.*	resilience	−0.88
$CaCO_3$/protein	*vs.*	$\frac{\text{opening moment}}{\text{g of shell}}$	0.37
Glycine/1000	*vs.*	resilience	0.94
Glycine/1000	*vs.*	$\frac{\text{opening moment}}{\text{g of shell}}$	−0.09
Cystine/2	*vs.*	resilience	−0.86
Cystine/2	*vs.*	$\frac{\text{opening moment}}{\text{g of shell}}$	0.23

between chains. The low proline content assures that the peptide chains will not be prevented (by this amino acid, at any rate) from hydrogen bonding with one another. While the evidence at this point is circumstantial, it does appear that based on comparative amino acid analysis alone, the ligaments of the pectens are most suited to a rubber-like elasticity. Hydrogen bonding, such as could be present in *A. irradians* or *P. magellanicus*, would presumably limit the freedom of movement of the chains and reduce the chances of true rubber-like elasticity. The absence of lipid and the low concentration of carbohydrate are indicative of all the bivalve ligament studies (Table I).

Calcium carbonate commonly occurs in living systems as calcite, aragonite, and, less commonly, vaterite. These are distinct minerals with different physical properties, such as unit cell dimensions, effect on polarized light, stability, and even solubility. This last difference is the basis for distinguishing the different phases by staining techniques (Feigl, 1954). This approach is practical for large amounts of mineral such as is found in the molluscan shell, but it was found inappropriate for the calcium carbonate of the ligament and the powder method of X-ray diffraction was employed.

Stenzel (1962) found aragonite in the resilium of oysters and Bevelander and Nakahara (1969) reported that the inner ligament of *Mytilus edulis* and *Pinctada radiata* contained only long, needled shaped, single aragonite crystals. The ubiquity of aragonite in bivalve ligament, independent of the crystalline phase in the shell, suggests that certain of the physical properties of aragonite may be important to the mechanical operation of the ligament.

The Pectinidae which have the weakest ligaments also have the most resilient ligaments when based on the shell weight (Table III and Figure 4). Perhaps, the most complicated mechanical properties are observed in *Mytilus edulis* which appears to have the strongest abduction system to any of the species examined. The inner and outer ligaments are reduced; however, the periostracum of *Mytilus* is greatly elongated in the anteroposterior direction. Trueman (1960a) measured considerable extension and contraction of the periostracum. The elaborated development of and the mechanical properties of the periostracum may account for the combination of resilience and strength. The mechanism by which this peculiar geometry affects strength and resilience is not apparent and needs further study.

The *Mytilus* abduction system is contrasted by *Ensis directus* and *Crassostrea virginica*, both of which are relatively weak abduction systems of low resilience (Table III and Figure 4). The burrowers are not outstanding in either strength or resilience. There is no clear trend relating life style to ligament strength or resilience in the species examined; however, it is notable that the swimmers are the most resilient and also the weakest.

In an attempt to relate the physical properties of the ligament with its structure and composition, the correlation coefficients were calculated for several sets of parameters as shown in Table IV. Only six of the experimental animals were included in the calculation; specimens of *Mercenaria mercenaria* and *Ensis directus* were excluded because these species have abducting external ligaments. Three sets of parameters show good correlation and resilience is involved in all three. This data is shown graphically in Figures 5, 6, and 7.

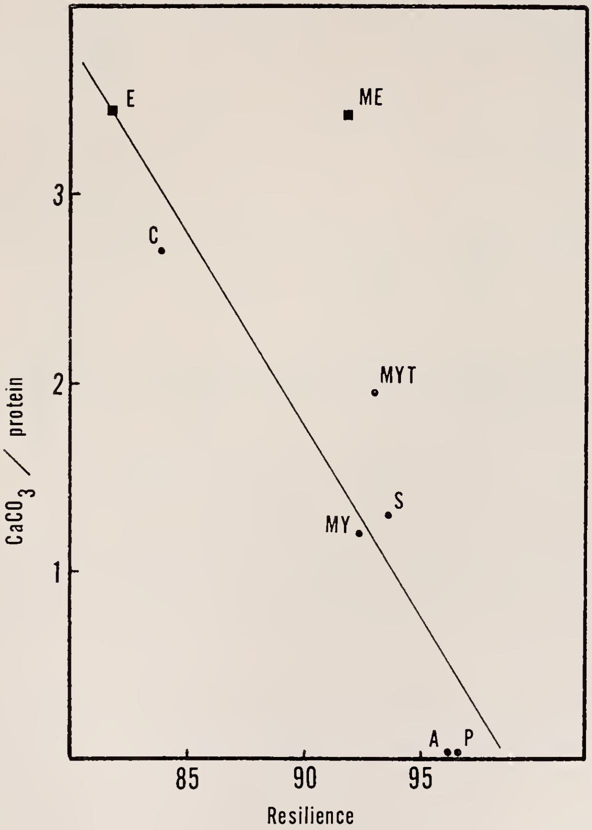


FIGURE 5. The $CaCO_3$/protein ratio as a function of resilience. Specimens of *Ensis* and *Mercenaria* were excluded in the calculation of the regression line—see text for explanation. Abbreviations as in Figure 4. The correlation coefficient is —0.88.

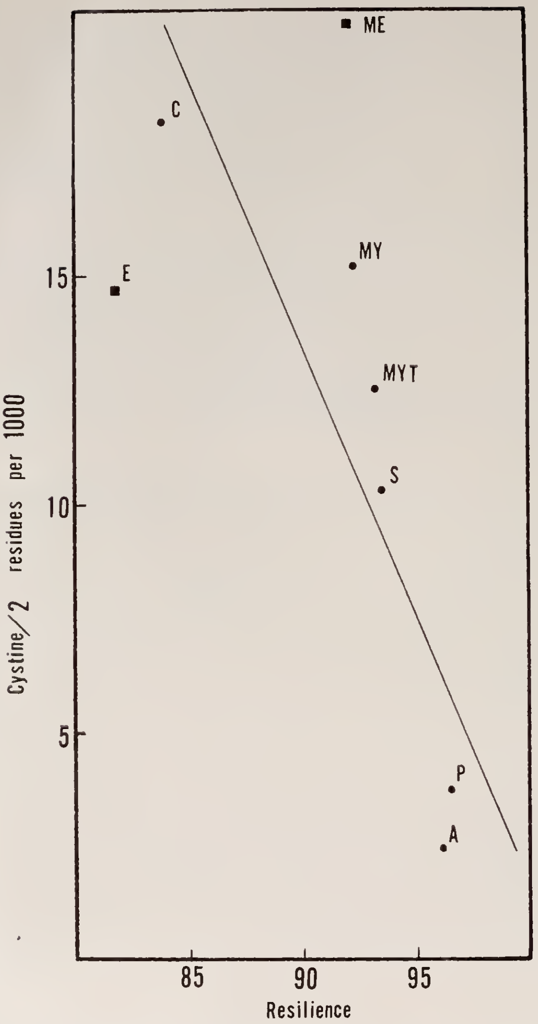


Figure 6. Cystine/2 residues per thousand as a function of resilience. Specimens of *Mercenaria* and *Ensis* were excluded in the calculation of the regression line—see text for explanation. Abbreviations as in Figure 4. The correlation coefficient is —0.86.

It appears that the aragonite crystals may interfere with the ability of the ligament to recover from compression (Figure 5). Similarly, larger concentrations of cystine/2 are correlated with lower resilience (Figure 6). This inverse relationship may be due to the stiffening of the ligament by the cross-linking of protein chains. Glycine concentration is directly related to resilience (Figure 7). Apparently the more closely the ligament approaches polyglycine, the more efficiently it can function. It is notable that the strength of the ligament (opening moment/g of shell) is not well correlated with any of the parameters considered in Table IV.

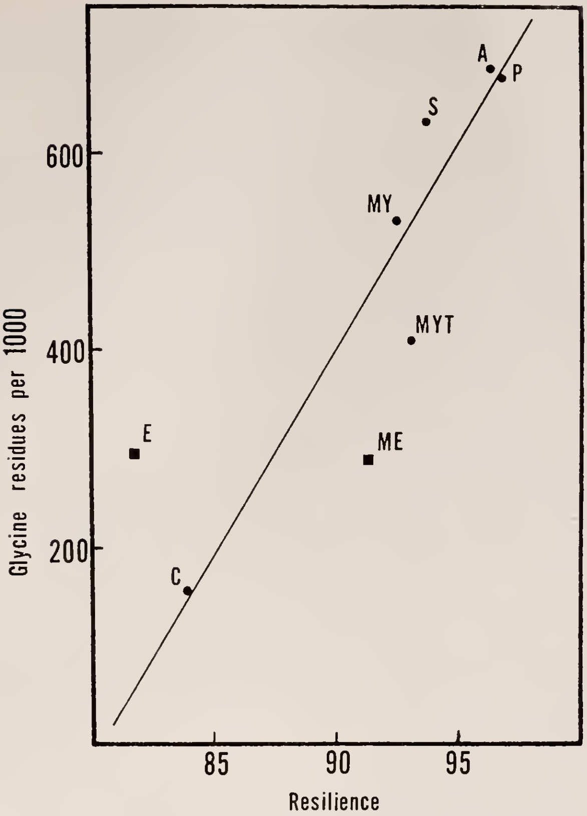


FIGURE 7. Glycine residues per thousand as a function of resilience. Specimens of *Mercenaria* and *Ensis* were excluded in the calculation of the regression line—see text for explanation. Abbreviations as in Figure 4. The correlation coefficient is 0.94.

The inverse relationship of cystine/2 and resilience, and the direct relationship of glycine concentration and resilience may support the theory of long thermally agitated protein chains acting with rubber-like elasticity. The greater the glycine concentration, the more the chains become rubber-like. Lower cystine/2 concentrations may indicate fewer cross-linkages and less restrictions on the chains, again suggesting a more rubber-like form. The possibility of β-type protein structure in the inner ligaments (as shown by X-ray diffraction) argues against rubber-like elasticity, as it requires that the chains be bound together in a backbone.

The ubiquity of the aragonite phase of $CaCO_3$ in ligaments is not readily explainable. It may be that the rod-like nature of aragonite, as opposed to calcitic spherules, provide a mechanical "firmness" similar to re-enforcing rods in concrete. It is notable that the essentially noncalcified ligaments of *Aequipecten* and *Placopecten* are among the weakest; however, the heavily calcified ligament of *Crassostrea virginica* is also relatively weak. The structural analysis of the inner ligament of *Spisula* will be considered in another publication.

We wish to thank the members of the Supply Department at the Marine Biological Laboratory for their prompt and efficient delivery of living material.

Summary

1. The bivalve ligament protein has a high percentage of glycine.

2. The absence of hydroxyproline and hydroxylysine in the ligament and the lack of a wide angle diffraction pattern indicate that the ligament is not collagen.

3. The ligament contains more sulfur amino acids—methionine and cystine/2—than other structural proteins.

4. Only the aragonite phase of calcium carbonate has been observed in association with the ligaments.

5. Members of the family Pectinidae have the weakest and most resilient ligaments.

6. Resilience is inversely correlated with $CaCO_3$ and cystine/2 concentration while glycine is directly correlated with resilience.

7. The strength factor (opening moment/gram of shell) is distinct from resilience and does not correlate with any of the parameters examined.

8. Recovery from compression by inner ligament is probably mediated through easing of steric strains induced during compression.

LITERATURE CITED

Alexander, R. M., 1966. Rubber-like properties of the inner hinge-ligament of Pectinidae. *J. Exp. Biol.*, **44**: 119–130.

Alexander, R. M., 1968. *Animal mechanics.* University of Washington Press, Seattle, Washington, 346 pp.

Andersen, S. O., 1967. Isolation of a new type of cross link from the hinge ligament protein of molluscs. *Nature*, **216**: 1029–1030.

Beedham, G. E., 1958. Observations on the non-calcareous component of the shell of the lamellibranchia. *Quart. J. Microsc. Sci.*, **99**: 341–357.

Bevelander, G., and H. Nakahara, 1969. An electron microscope study of the formation of the ligament of *Mytilus edulis* and *Pinctada radiata*. *Calcified Tissue Res.*, **4**: 101–112.

Campbell, J. W., 1960. Nitrogen and amino acid composition of three species of Anoplocephalid cestodes: *Moniezia expansa, Thysanosoma actinioides* and *Cittotaenia perplexa*. *Exp. Parasitol.*, **9**: 1–8.

Campbell, J. W., S. P. Bonner, and T. W. Lee, 1968. Enzymes of arginine and urea metabolism in invertebrates. Pages 1–23 in G. A. Kerkut, Ed., *Experiments in physiology and biochemistry 1.* Academic Press, New York.

Chibnall, A. C., J. L. Mangan, and M. W. Rees, 1958. Studies on the amide and c-terminal residues in proteins. 2. The ammonia nitrogen and amide nitrogen of various native protein preparations. *Biochem. J.*, **68**: 111–114.

Christian, G. D., and F. J. Feldman, 1970. *Atomic absorption spectroscopy: applications in agriculture, biology and medicine.* Wiley-Interscience, John Wiley and Sons, New York, 512 pp.

Dall, W. H., 1889. On the hinge of the pelecypods and its development, with an attempt toward a better subdivision of the group. *Amer. J. Sci.*, **138**: 445–462.

Dall, W. H., 1895. Tertiary mollusks of Florida. *Wagner Free Institute of Science, Transactions*, **3**: 484–566.

Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.*, **28**: 350–356.

Feigl, F., 1954. *Spot tests I. Inorganic applications.* Elsevier Publishing Company, New York, 518 pp.

Folch, J., I. Ascoli, M. Lees, J. A. Meath, and F. N. LeBaron, 1951. Preparation of lipid extracts from brain tissue. *J. Biol. Chem.*, **191**: 833–841.

Galtsoff, P. S., 1964. The American oyster *Crassostrea virginica* (Gmelin). *U. S. Fish Wildlife Serv. Fish. Bull.*, **64**: 1–480.

Hare, P. E., 1963. Amino acids in the proteins from aragonite and calcite in shell of *Mytilus californianus*. *Science*, **139**: 216–217.

Harrington, W. F., and P. H. Von Hippel, 1961. The structure of collagen and gelatin. *Advan. Protein Chem.*, **16**: 1–127.

Jackson, R. T., 1890. Phylogeny of the Pelecypoda. *Memoirs of Boston Soc. Natur. Hist.*, **4**: 277–400.

Jackson, R. T., 1891. The mechanical origin of structure in the pelecypods. *Amer. Natur.*, **25**: 11–21.

Kelly, R. E., and R. V. Rice, 1967. Abductin: a rubber-like protein from the internal triangular hinge ligament of pecten. *Science*, **155**: 208–210.

Lang, C. A., 1958. Simple microdetermination of Kjeldahl nitrogen in biological materials. *Anal. Chem.*, **30**: 1692–1694.

Owen, G., E. R. Trueman, and C. M. Yonge, 1953. The ligament in the Lamellibrancia. *Nature*, **171**: 73–75.

Partridge, S. M., 1962. Elastin. *Advan. Protein Chem.*, **17**: 227–302.

Piez, K. A., 1961. Amino acid composition of some calcified proteins. *Science*, **134**: 841–842.

Russell Hunter, W., and D. C. Grant, 1962. Mechanics of the ligament in the bivalve *Spisula solidissima* in relation to mode of life. *Biol. Bull.*, **122**: 369–379.

Seifter, S., and P. M. Gallop, 1966. The structural proteins. Pages 153–458 in H. Neurath, Ed., *The proteins, 4*. Academic Press, New York.

Seligson, D., and H. Seligson, 1951. A microdiffusion method for the determination of nitrogen liberated as ammonia. *J. Lab. Clin. Med.*, **38**: 324–330.

Stenzel, H. B., 1962. Aragonite in the resilum of oysters. *Science*, **136**: 1121–1122.

Travis, D. F., C. J. Francois, L. C. Bonar, and M. J. Glimcher, 1967. Comparative studies of the organic matrices of invertebrate mineralized tissues. *J. Ultrastruc. Res.*, **18**: 519–550.

Trueman, E. R., 1942. The structure and deposition of the shell of *Tellina tenuis*. *J. Roy. Microsc. Soc.*, **62**: 69–92.

Trueman, E. R., 1949. The ligament of *Tellina tenuis*. *Proc. Zool. Soc. London*, **119**: 717–742.

Trueman, E. R., 1950a. Observations on the ligament of *Mytilus edulis*. *Quart. J. Microsc. Sci.*, **91**: 225–235.

Trueman, E. R.. 1950b. Quinone-tanning in the Mollusca. *Nature*, **165**: 397–398.

Trueman, E. R., 1951. The structure, development, and operation of the hinge ligament of *Ostrea edulis*. *Quart. J. Microsc. Sci.*, **92**: 129–140.

Trueman, E. R., 1953. Observations on certain mechanical principles of the ligament of *Pecten*. *J. Exp. Biol.*, **30**: 453–467.

Trueman, E. R., 1954. Observations on the mechanism of the opening of the valves of a burrowing lamellibranch, *Mya arenaria*. *J. Exp. Biol.*, **31**: 291–305.

Trueman, E. R., 1964. Adaptive morphology in paleoecological interpretation. Pages 45–74 in J. Embrie and N. Newell, Eds., *Approaches to paleoecology*. Wiley and Sons, New York.

Trueman, E. R., 1966. Bivalve mollusks: fluid dynamics of burrowing. *Science*, **152**: 723–725.

Weis-Fogh, T., 1961. Molecular interpretation of elasticity of resilin, a rubber-like protein. *J. Mol. Biol.*, **3**: 648–667.

Willis, J. B., 1960. The determination of metals in blood serum by atomic absorption spectroscopy I. Calcium. *Spectrochim. Acta*, **16**: 259–272.

Reference: *Biol. Bull.*, **151**: 182–199. (August, 1976)

NERVOUS CONTROL OF CILIARY ACTIVITY IN GASTROPOD LARVAE

GEORGE O. MACKIE, C. L. SINGLA, AND CATHERINE THIRIOT-QUIEVREUX

The Biology Department, University of Victoria, Victoria, B. C., Canada, and the Station Zoologique, Université de Paris VI, Villefranche-sur-Mer, 06230 France

Gastropod veligers have been called "the most spectacular of all molluscan larvae" (Fretter, 1967, p. 357), displaying many adult characteristics along with an elaborate and characteristically larval structure, the velum, which serves for locomotion and food collection (Fig. 1). Light microscopic observations (Carter, 1926, 1928; Werner, 1955; Thompson, 1959; Fretter, 1967) agree on the existence of muscle and nerve fibers radiating out across the velum from the head on either side, but these accounts differ in many matters of detail. Werner (1955) and Fretter (1967) describe a network of nerve cells whose cell bodies lie in the velum, but such cells do not figure in Carter's more detailed account (1926, 1928). Carter found only the ramifying branches of nerves originating in the brain. Fretter found no nerve connections between the brain and ciliated epithelium, but such connections are depicted by Carter (1926, 1928) and by Thompson (1959). The mass of radiating nerve fibers shown by Thompson somewhat resemble the radiating fibers called retractor muscle fibers by Fretter. Here and elsewhere there may have been a failure to distinguish the two fiber types clearly. Thompson and Werner indicate that there are local, velar muscle cells, as well as muscle fibers entering the velum from the body of the larva. Allowing for differences between species, there still appear to be several important points in need of resolution.

The present study is concerned largely with elucidating the neuromuscular relationships in the velum, and with tracing the motor innervation of the ciliated cells of the preoral band, which are responsible for locomotion. Carter (1926, 1928) claimed that the ciliated cells were innervated and that the intermittent arrests of ciliary beating which he described were due to nervous inhibition. This work has been justly cited as a classic in the field of ciliary control. The pharmacology of ciliary control in veligers has been explored in some depth (Koshtoyants, Buznikov and Manukhin, 1961; Buznikov and Manukhin, 1962; Korobtsov and Sakharov, 1971) but there has been no verification of Carter's key claim regarding neurociliary junctions and in fact Aiello (1974) was unable to confirm the existence of such junctions in T. E. Thompson's electron micrographs. Neurociliary synapses have also proved elusive in bivalve gills (Paparo, 1972) where similar histological relationships might otherwise be expected to prevail.

The present study supports Carter's findings on innervation and further provides new evidence from electrophysiological recordings regarding the ciliary control mechanism. Intracellular recordings from a post-veliger pteropod larva here provide evidence complementing the results with veligers.

Taken in conjunction with the Russian pharmacological work, these results show quite clearly that the preoral cilia are strictly controlled by the larval nervous system, and that the system is probably little or no less sophisticated that the ciliary control

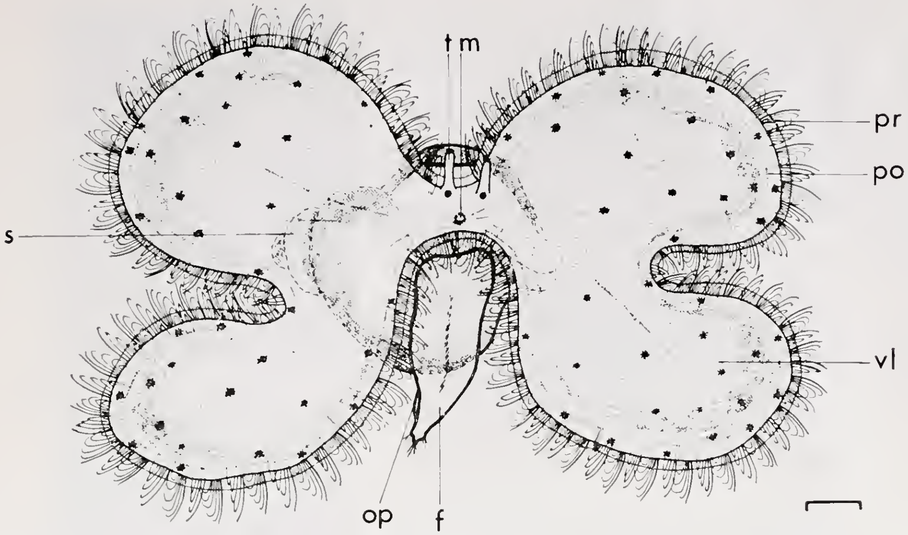


FIGURE 1. Veliger larva of *Mangelia*. Abbreviations are f, foot; m, mouth; op, operculum; po, postoral ciliated band; pr, preoral ciliated band; s, shell; t, tentacle; vl, lobe of velum; scale, 100 μ.

system in bivalve gills, currently the subject of active investigation in several laboratories (see reviews by Aiello, 1974; Jørgenson, 1975).

MATERIALS AND METHODS

Several species of veliger larvae, including both prosobranch and opisthobranch genera were investigated in this study, but the reported observations refer to *Mangelia nebula* (Montagu) and to the *Mangelia* sp. termed species C (Thiriot-Quiévreux, 1969), which were convenient to study, being larger and less prone to retract into their shells than most other prosobranch species. Polytroch larvae of the gymnosome pteropod *Pneumoderma atlanticum* (Oken) were also investigated. These post-veliger larvae lack a velum, but have three ciliated rings, one at the base of the head, one in the middle of the body and a third near the posterior end (Fol, 1875). The ciliated bands persist long after the appearance of adult organs and continue to serve for locomotion even after the wings are developed.

The larvae were retrieved from freshly collected plankton hauls in the bay of Villefranche-sur-Mer during the period January to May 1975. They were isolated in clean water and used for experiments within a day or two of collection.

Fine polyethylene tubes were used as suction holders, doubling as electrodes for stimulating and recording. Tubes of about 30μ internal tip diameter were chiefly used. Attachment of these electrodes stimulates the animal initially and causes contraction and ciliary arrest, but if the suction applied is not excessive, specimens soon relax and resume normal activity. For intracellular recordings, glass microelectrodes of 40–50 megOhms resistance were used in conjunction with a Medistor A 35 electrometer amplifier, with display on a Tektronix 5102 storage oscilloscope,

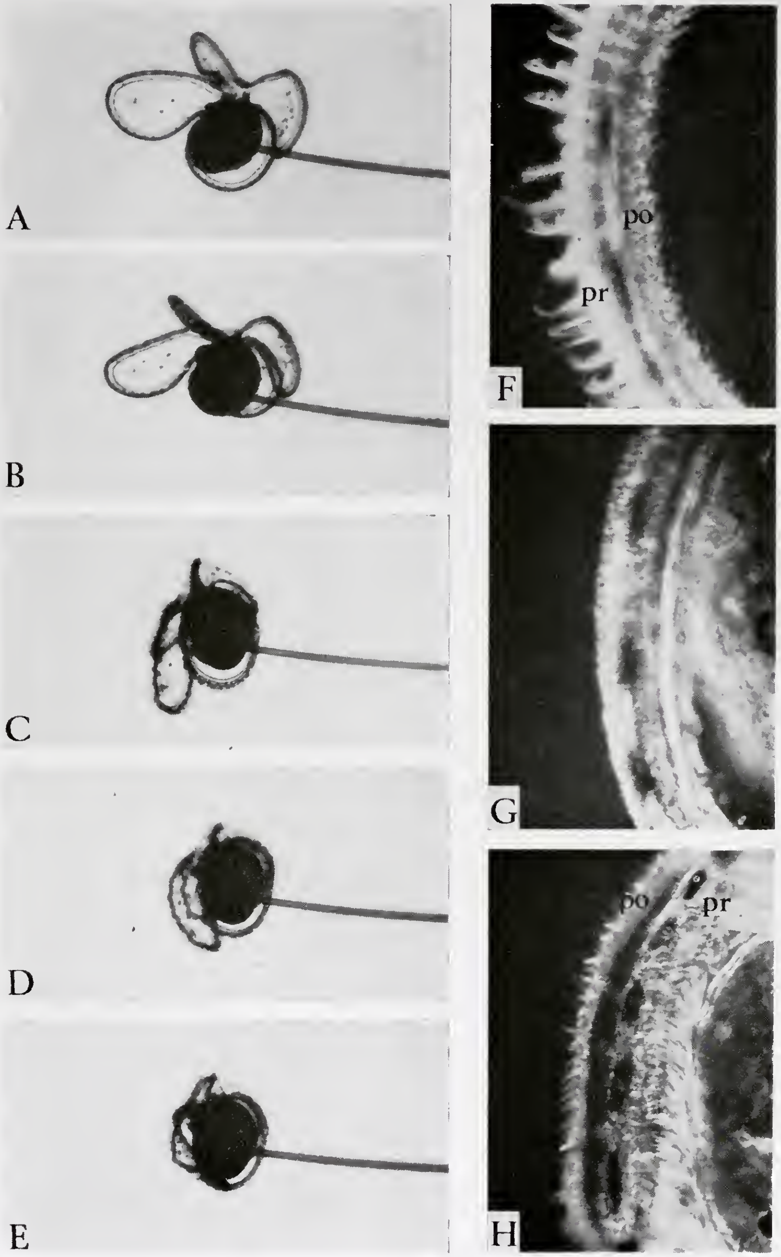


FIGURE 2. A–E, veliger attached to electrode, showing stages of recovery following a protective contraction. F–H, flash photos of velar margin showing the preoral (pr) cilia exhibiting the normal metachronal rhythm (F), arrested at start of power stroke (G) and curled inward aborally, allowing postoral (po) cilia to be seen at left hand edge(H). (See further in text.)

or a Grass 79C pen-writing oscillograph. A Grass S 44 stimulator was used for giving electrical shocks.

A Zeiss microscope equipped with bright-field, phase contrast and Nomarski interference optics was used for tracing the distribution of nerves and muscle fibers in the velum. A Zeiss Ukatron flash unit was used to photograph cilia in motion. Specimens were examined alive, either unstained or after staining with rongalit-reduced methylene blue. Portions of the velum to be examined were removed from larvae narcotised in sea water containing additional magnesium chloride to about 150 mM, sometimes with the addition of a small pinch of EGTA. Carter (1926) recommended nicotine as a good narcotic, but we have not found it to be as effective as magnesium for our purposes.

Electron microscopy was carried out on sections cut from Epon blocks of tissue fixed in 4% glutaraldehyde, postfixed in 2% osmium tetroxide, both buffered in cacodylate buffer. Uranyl acetate and lead citrate were used as stains, and sections were examined with a Philips EM 300.

RESULTS

General observations

Veligers with the velum expanded normally (Figs. 1 and 2A) show a continuous laeoplectic metachronism in the preoral (locomotory) ciliated band (Carter, 1926; Knight-Jones, 1954; Fretter, 1967). Contact with another solid object or with the surface film causes ciliary arrest, usually along with some degree of muscular contraction, depending on the intensity of the stimulus. A sufficiently light touch causes momentary ciliary arrest with little or no muscle contraction.

In nature, ciliary arrests would result in sinking and might be evoked by a variety of tactile, and perhaps chemical, stimuli (Fretter, 1967). The folding of the velar lobes around the shell (due to contraction of the intrinsic velar musculature) or withdrawal into the shell (due to contraction of retractor fibers) would doubtless assist sinking by reducing frictional resistance and would reduce the vulnerability of the velum to damage. However, as Carter (1926) noted, these responses sometimes occur "spontaneously," *i.e.*, in the absence of a stimulus apparent to the observer. The significance of such events in relation to vertical migration will be discussed further.

The sequence of stages shown in Figure 2A–E represents recovery following a strong contraction which fell short of retraction into the shell. In E the lobes are clasped tightly around the shell; the cilia are arrested. In D, the cilia are still arrested, but the lobes are starting to relax. In C the cilia are straightening out from their recurved posture and are starting to beat irregularly. In B, metachronism is established and muscular relaxation is almost complete. The crinkling of the velar border seen in D is presumably due to the tight contraction of the marginal muscle fibers which underlie the preoral band.

Flash photos of the velar margin, viewed from the aboral side, are shown in Figure 2F-H. In F, the preoral and postoral bands are showing normal metachronism. In G, a weak stimulus has caused arrest of the cilia of the preoral band. The cilia are flexed aborally into a position corresponding to the beginning of the power stroke. A stronger stimulus (H) causes not only ciliary arrest, but a muscle

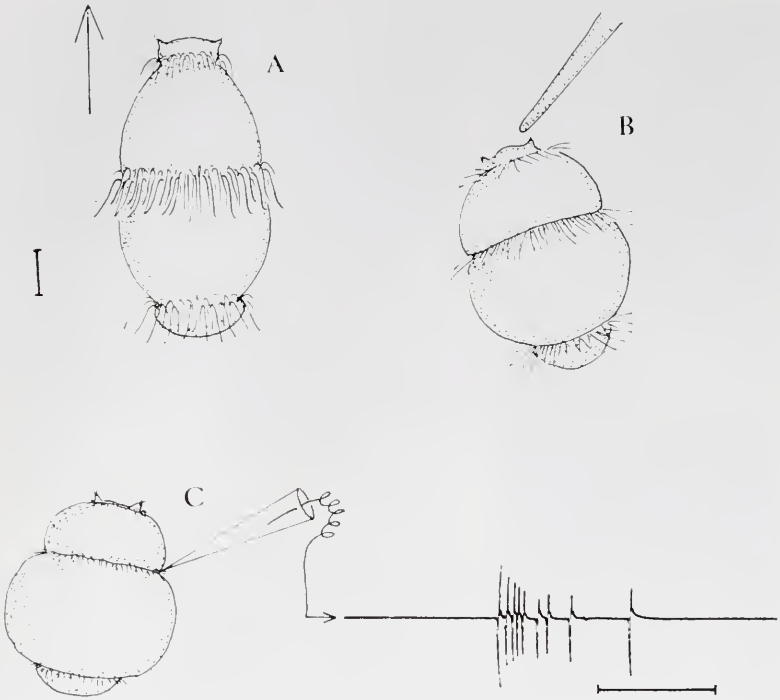


FIGURE 3. Polytroch larva of *Pneumoderma atlanticum*, swimming normally (arrow) in A, contracted with arrested cilia following brief contact with a probe (B) and more strongly contacted (C). Electrical record in C shows ciliary arrest potentials recorded extracellularly from a ciliated band. Size scale equals 100μ; time scale, 1 sec.

response: the curling aborally of the velar margin and preoral band. This brings the postoral band into view at the outer edge (left), the arrested cilia of the preoral band being seen now on their right.

We have observed no synchronized arrests in ciliated cells other than those of the preoral bands, which agrees with Thompson's (1959) findings in *Archidoris*. The cilia of the postoral bands and food groove continue normal metachronal beating when the preoral bands are arrested. Thus it is not necessary to assume, as does Richter (1973), that feeding is impossible during arrest of the locomotory cilia.

Pneumoderma larvae crawl on the bottom of their dishes or swim freely in the water by means of their ciliated bands, the cilia showing laeoplectic metachronism, like those of the veligers. Figure 3A shows a young larva extended and swimming normally in the direction shown by the arrow. A touch with a glass probe (B) causes the cilia to stop beating at the end of the power stroke or to lose their metachronism, beating weakly and irregularly. Ciliary arrest may be accompanied by some degree of muscle contraction, typically a symmetrical shortening of the whole body, which causes the sides to bulge and the cilia to be partially retracted into circular furrows. In a stronger response, such as the one shown in C, the cilia would be totally arrested and retracted until only the tips showed. These responses are thought to be protective in nature as they result in rounding of the body, cessation of forward movement, sinking, and shielding of the cilia. Spontaneous ciliary arrests of variable duration are seen here, as in the veligers.

Older *Pneumoderma* larvae swim by the action of their cilia aided by occasional bouts of wing undulation. Stimuli which provoke ciliary arrest and body shortening also cause the wings to retract, though retraction can occur by itself, without relation to the general protective response. Other independent responses include unilateral body flexions and extrusion of the buccal mass. Cessation of the heart beat is seen during strong contractions, but whether this represents nervous inhibition or is a secondary effect of contraction, mediated perhaps by fluid pressure, has not been determined.

Isolation of ciliated bands from the central nervous system

Ciliary metachronism is quickly re-established in velar lobes cut from *Mangelia* larvae. By contrast, the distal fragments remaining attached to the head take a long time to expand and to start beating again. It is difficult to provoke ciliary arrest in pieces whose connections with the brain have been cut. Small areas may show local arrest, but there is little if any spread to adjacent regions.

Bisection of a *Pneumoderma* larva by a transverse cut just anterior to the middle ciliated band resulted in the loss of spontaneous coordinated ciliary arrests in the posterior half, and it became impossible to provoke arrests by strong tactile or electrical stimulation.

It seems that severing the connections with the brain abolishes the transmission pathway for the arrest response in both types of larvae.

Electrical correlates of ciliary arrest

Electrodes attached to the velar surface in *Mangelia* show no potential changes during normal ciliary activity, not even during the slight muscular twitches and flexions which velar lobes perform. As soon as there is a ciliary arrest, whether or not muscles contract at the same time, the electrode picks up one or a series of large (1–3 mV) potentials (Fig. 4A). Comparable events are recorded from *Pneumoderma* larvae (Fig. 3C). A single brief arrest (little more than a momentary interruption in the metachronal beating) is represented by a single potential in the electrical record (Fig. 4B, at point 1). A sustained series of potentials represents a sustained arrest, as in Figure 3C and in Figure 4B following point 2. If the interval between the potentials is long enough, as in Figure 4A toward the end of the burst, each potential can be correlated visually with a discrete postural shift in the arrested, or weakly beating, cilia. Spontaneous bursts (Fig. 4B) resemble bursts evoked by tactile or electrical stimulation.

The presumption that the potentials represent ciliary arrests rather than muscle twitches is supported by the observations that muscle twitches without arrests are not accompanied by potentials, while arrests without twitches are accompanied by these electrical signals. It is inherently unlikely that the potentials represent nerve action potentials, nerves being small, deep-lying, presumably well-insulated structures; there would be no precedent for recording their activity at the surface, even less as pulses in the millivolt range. As in echinoderm larvae (Mackie, Spencer

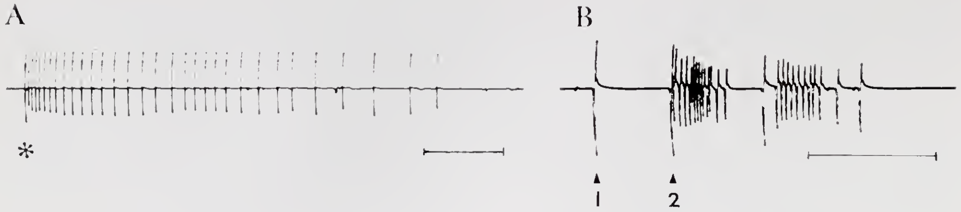


FIGURE 4. Ciliary arrest potentials recorded extracellularly from *Mangelia* (A) and *Pneumoderma* (B). In A, a 1 msec electrical stimulus close to threshold strength (asterisk) evoked a long series of potentials. In B, a light touch (1) evoked a single arrest potential, a stronger touch (2) a series. The final burst was spontaneous. Time scales equal 0.5 sec (A) and 1.0 sec (B).

and Strathmann, 1969), larvaceans (Galt and Mackie, 1971), and ascidians (Mackie, 1974; Mackie, Paul, Singla, Sleigh and Williams, 1974), it would here be proposed that the potentials accompanying ciliary arrests (reversals in larvaceans) represent the summed activity of a large number of simultaneously depolarizing ciliated cells. The resulting bioelectric currents give rise to potential changes which can be picked up all over the body surface.

Proof of the origin of the potentials from ciliated cells has been obtained by inserting microelectrodes into visually identified ciliated cells, which is feasible in young *Pneumoderma*, thanks to the large size of the cells and the feebleness of the muscular movements. As the accompanying records show (Fig. 5A, B) the events interpreted as ciliary arrest potentials in the extracellular (upper) record are revealed by the intracellular (lower) record as 50 mV depolarizations rising quickly to a peak approximately at zero potential and decaying slowly, over about 400 msecs. As shown in Figure 5B there is little summation of consecutive spikes, even when the interval between them is less than 50 msec, so each spike can be considered as an all-or-none event. The extracellular records show distortion of the wave form due to capacitance in the system. The second of two events occurring in close succession characteristically appears attenuated in the extracellular records (Fig. 5B), since the cells are already partially depolarized from the preceding potential at the time of onset of the second. The attenuation of the potentials comprising high frequency bursts, for example in Figure 4B, is explicable on the same basis.

TABLE I

Comparison of metazoan ciliary arrest potentials, measured intracellularly.

	Resting potential (millivolts)	Amplitude (millivolts)	Rise time (milliseconds)	Decay time (milliseconds)
Corella[1]	35–40	45–50	40	1500
Mytilus[2]	60	<20	30	170
Pneumoderma[3]	50	50	15	400

[1] Mackie *et al.* (1974).
[2] Murakami and Takahashi (1975).
[3] Present paper.

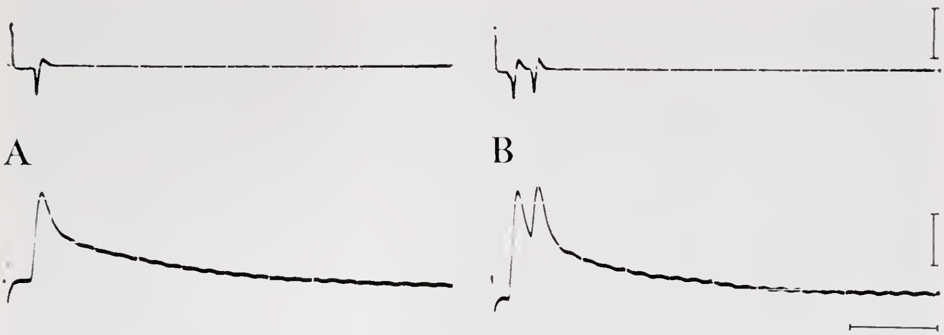


FIGURE 5. *Pneumoderma:* simultaneous extracellular (upper) and intracellular (lower) records of ciliary arrest potentials. A shows a single arrest potential, B two such potentials 45 msec apart. Time scale equals 100 msec; amplitude scales, 3 mV (extracellular) and 20 mV (intracellular).

The duration of ciliary arrest probably corresponds fairly closely to the duration of depolarization of the cells, but this relationship has not been accurately determined. To do so would require simultaneous monitoring of the ciliary beating with a photomultiplier, a technique used for the first time in a metazoan by Murakami and Takahashi (1975).

During normal ciliary activity the membrane potential is steady and unwavering as in the ascidian *Corella* (Mackie *et al.*, 1974) and in *Mytilus* (Murakami and Takahashi, 1975).

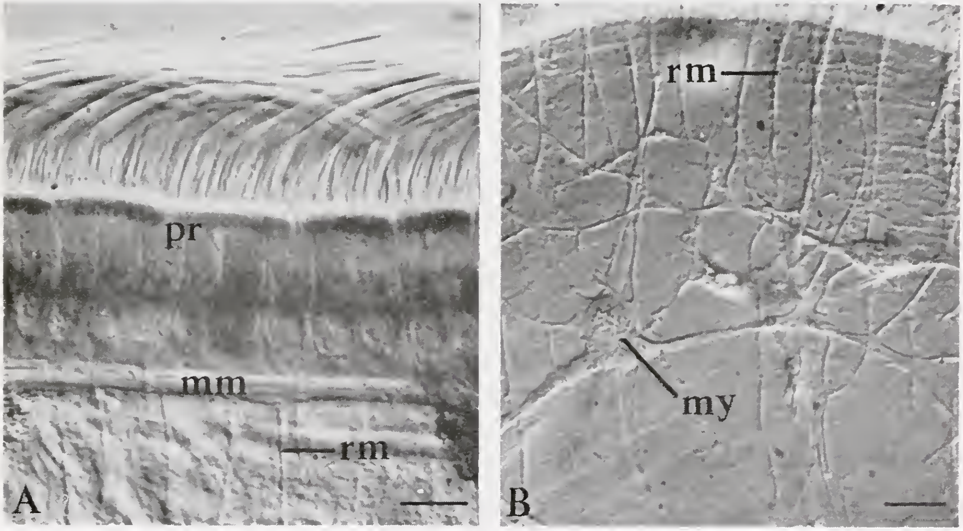


FIGURE 6. *Mangelia:* interference contrast photographs of the living velum. A., velar margin showing preoral band, B., area adjacent to margin. Abbreviations are mm, marginal muscle band; my, myocyte; pr, ciliated cells; rm, radial muscle strand; scales, 20 μ.

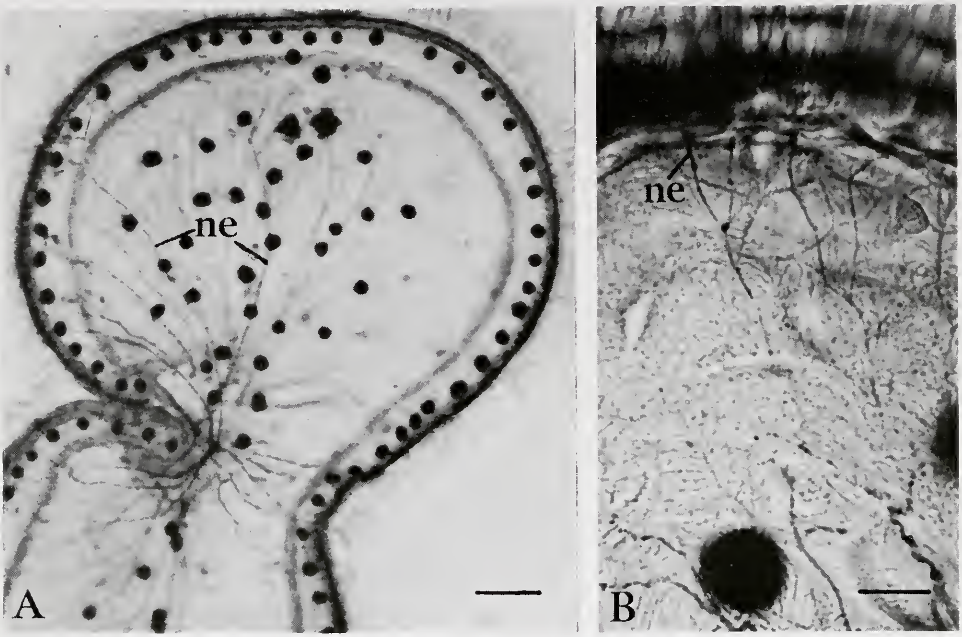


Figure 7. *Mangelia:* velum stained with methylene blue: A., whole lobe; B., an enlarged area near the margin. ne represents nerve process; scales, 50 μ in A, 10 μ in B.

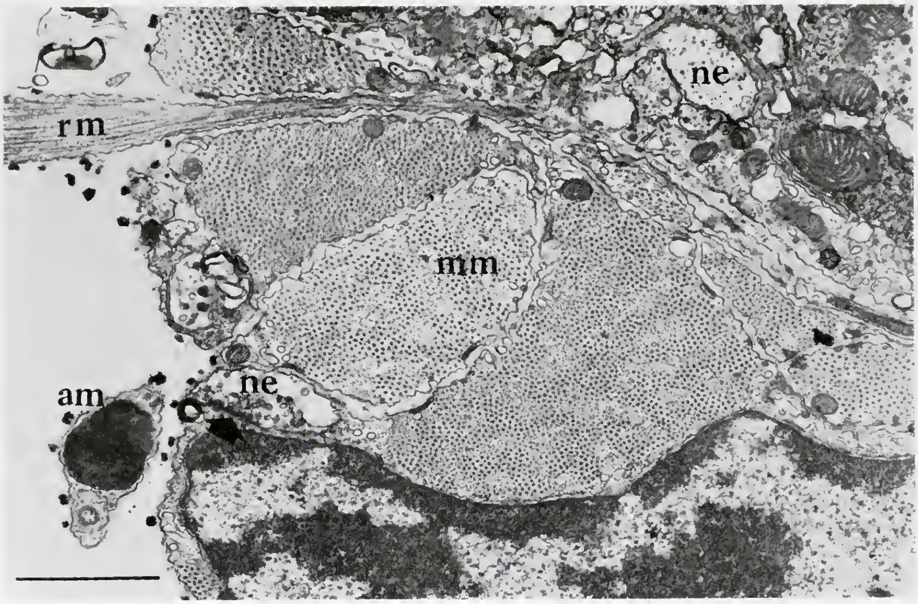


Figure 8. *Mangelia:* section of velar margin in the region of the marginal muscle band (see Fig. 10). Abbreviations are am, process of amoebocyte; ne, nerve process; mm, marginal muscle fiber; rm, radial muscle fiber. Arrow shows a neuromuscular junction; scale, 1.0 μ.

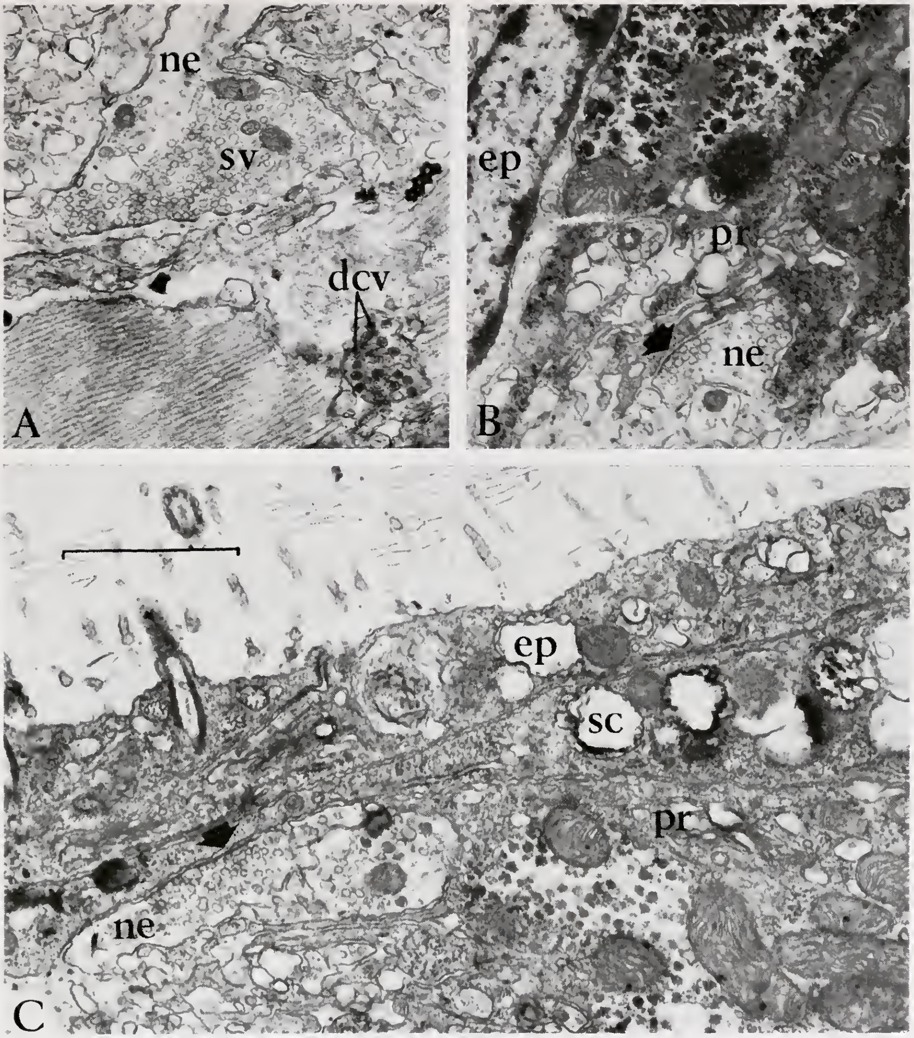


FIGURE 9. *Mangelia:* sections of velar margin in region of marginal muscle band showing two sorts of nerve vesicles (A), ciliated cell of preoral band (B) and supporting cell (C) (see Fig. 10). Abbreviations are dcv, nerve process with dense-cored vesicles; ep, epithelial cell; ne, nerve process; pr, ciliated cell; sc, supporting cell; sv, small clear vesicles. Synapses (arrows) are characterized by thickened presynaptic membranes and aggregations of small clear vesicles. Scale equals 1.0 μ.

Morphology and fine structure

The following account refers chiefly to *Mangelia nebula.*

Preoral band. The appearance of the living cells under the interference microscope is shown in Figure 6A. Further details of the ciliated cells as seen by light microscopy are given by Carter (1926, 1928) and Fretter (1967). The

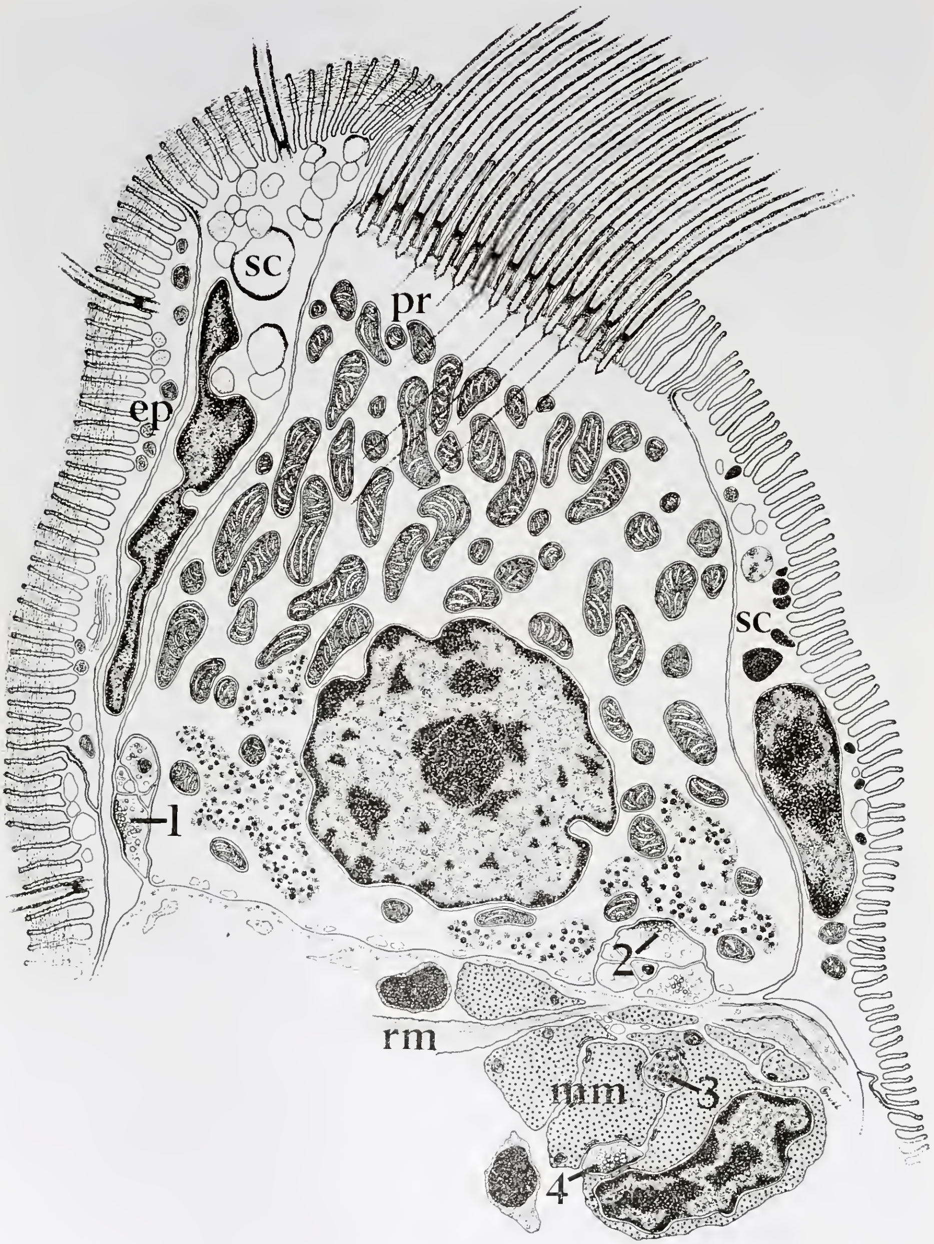


FIGURE 10. *Mangelia:* drawing summarizing histological relationships seen in radial sections through the preoral ciliated band. The ciliated cell (pr) contains large numbers of mitochondria and glycogen islets. Supporting cells (sc) lie on either side. Epithelial cells (ep) cover the velum, abutting on the supporting cells. All these cells bear microvilli. On the left, which is the side of the "food groove" (Fretter, 1967), the microvilli are embedded in a fibrillar mesh. The marginal muscle bundle (mm) lies toward the aboral side, with radial muscle fibers

major features of the cells as revealed by electron microscopy are summarized in Figure 10 and need little additional description. The cells are distinguished by a complex ciliary apparatus, by large numbers of mitochondria, and by deposits of granular material, resembling the pools of glycogen rosettes (α-glycogen) seen in *Helix* (McGee-Russell, 1968) and many other molluscs (Fig. 9 B, C). They receive synapses from local neurites which run in bundles within folds of the basal membrane (Fig. 9B). They are flanked on oral and aboral sides by supporting cells which are themselves partially clad in epithelial cell processes. Both the latter cell types are ciliated, but the cilia are short and far apart rather than being long and bunched together into compound units like those of the locomotory ciliated cells. All three cell types bear microvilli. On the food groove side (left in Fig. 10) a felt-like mass of layered fibrils is interspersed among the microvilli (Fig. 9C).

The supporting cells contain granules of amorphous, electron-dense material of unknown composition. They are innervated (Fig. 9C), which suggests that release of the product may be under nervous control. The epithelial cells also contain secretion bodies, perhaps mucus, but receive no synapses. All three cell types are joined by septate desmosomes near their outer edges.

Muscle layout. In addition to the velar retractor muscle system (Fretter, 1967, 1972), there is a local muscle system, corresponding to Fretter's "intrinsic muscles," consisting of the processes of myocytes which lie wholly within the velum. This "muscle net" consists of branching myocytes whose processes intermingle with the retractor fibers; the net lies on both sides of the velum and is specialized into a thick band, the marginal muscle, which runs circularly around the edge beneath the preoral band, toward the aboral side (Figs. 6A and 8). Contraction of this band probably causes the aboral inflexion and crinkling of the velar edge noted earlier as a response to stimulation. Radially arranged fibers deriving both from the retractor system and from myocytes in the muscle net run up to and around the marginal muscle. Their role would be to control general posture and curvature of the velum and to fold it when it is withdrawn.

The distribution of myocytes comprising the intrinsic musculature of the velum is highly organized and consistent within a given species, but varies widely between different species. Part of this muscle net is shown in Figure 6B, from the aboral side of the velum. The cells are readily distinguishable from amoebocytes. The latter have shorter processes and often lie completely separated from other amoebocytes. Their cell bodies show a very irregular and variable appearance and are generally less compact than those of myocytes. They are found in the blood spaces as well as near the surface epithelia. The myocytes might be confused with multipolar neurons, but their processes are more numerous, thicker, and less delicate than typical nerve net neurites and more prone to adhere together. Their cell bodies are less regular and compact, and no part of the cell shows an affinity for methylene blue in preparations where nerve processes of central origin are well stained. On the positive side, electron microscopy shows nucleated cell profiles with processes

---

(rm) passing out toward oral and aboral surfaces. Nerve elements are numbered as follows: 1, process synapsing with a supporting cell; 2, process synapsing with ciliated cell; 3, process containing dense-cored vesicles; and 4, process synapsing with a myocyte.

containing muscle filaments in the regions where myocytes are seen by light microscopy.

Under the electron microscope (Fig. 8) the muscle fibers, both in the local net and in the retractor strands, are seen to be highly differentiated structures with thick and thin filaments and small flattened sub-sarcolemmal cisternae. They are often seen to be associated with processes of amoebocytes. Neuromuscular synapses are frequently seen in the marginal muscle band.

Innervation. In the intact, living velum stained with methylene blue, nerves can be seen running from the point of attachment beside the head out across the velum in all directions (Fig. 7A). The prominent dark structures in this figure are pigment cells, which do not appear to be innervated. Only rarely are results sufficiently good to allow the fine terminal nerve branches to be selectively stained; but here and there this happens, and nerve terminals can be followed right into the marginal muscle band and to the bases of the ciliated cells (Fig. 7B). No evidence was found for a general velar nerve net (i.e. interconnected processes of cells with cell bodies dispersed across the velum), although there is a small group of local neurons on the aboral side near the head on either side.

While we agree with Carter's account on most points, we have only occasionally seen nerve endings running up between the ciliated cells. Generally they go no further than the bases of these cells. Under the electron microscope, neurites were found mingling with muscle fibers and enveloped in pockets within the bases of the ciliated cells. The innervation is rich and synapses are abundant.

Two sorts of vesicles are seen in the neurites, small clear vesicles with diameters ranging between 335 and 560 Å, and larger dense cored vesicles with diameters 560 to 835 Å (Fig. 9A). The two sorts are usually well segregated into different neurites.

Synapses are recognized by the usual criteria for gastropods (Amoroso, Baxter, Chiquoine and Nisbet, 1964), *viz,* thickened junctional membranes and massed, small, clear vesicles. Dense cored vesicles are rarely found at synapses.

As already noted, synapses are made with three different cell types: muscle cells, ciliated cells and supporting cells. The epithelial cells are not innervated. No nerve endings identifiable as sensory terminations have been spotted. The ciliated cells of the postoral (feeding) band do not appear to be innervated, although nerve bundles lie near their bases. This point needs further study and verification.

All the foregoing remarks in this section have referred to *Mangelia* veligers. The fine structure of *Pneumoderma* larvae has not been examined in as much detail, but it can be stated that the locomotory ciliated cells in this species are also richly innervated, receiving synapses whose appearance under the electron microscope closely resembles that of the synapses in *Mangelia.*

DISCUSSION

The evidence presented here confirms the findings of Carter (1926, 1928) regarding the existence of nerve fibers running from the brain to the velar preoral ciliated band. We cannot yet say how many ciliated cells are supplied by branches of the same nerve fiber, but there is clearly a very rich innervation; and in all probability each cell receives at least one nerve ending. There is no need therefore to assume extensive spread of excitation within large populations of ciliated cells, as in ascidian stigmata (Mackie *et al.*, 1974), although limited local spread might still occur. Motokawa and Satir (1975) report the slowly spreading arrest of ciliary beating in *Mytilus* following localized damage by laser irradiation, confirming earlier work on *Elliptio*. These spreading arrests are probably electrotonically mediated by current flow through gap junctions between the ciliated cells.

Nudibranch veligers gain the ability to arrest their cilia only after the innervation is established (Buznikov and Manukhin, 1962). It is shown here that separating the ciliated epithelium from the brain permanently abolishes coordinated ciliary arrests. In the ascidian *Corella* by contrast isolated bits of gill continue to exhibit arrests, showing that a conduction system and pacemakers are developed on the local level (Mackie *et al.*, 1974). Contrary to some earlier reports, we have found no evidence for a local nerve net in the velum, but only the nerve fibers which come from the brain.

At the fine structural level we have been able consistently to show neurociliary synapses both in veligers and gymnosome larvae. These appear to be the first neurociliary synapses described for a mollusc. Paparo (1972) has found a rich innervation in bivalve gills but no synapses. In annelid trochophore larvae, neurociliary and neuromuscular synapses similar to those described here are reported (Holborow, 1971). In the trochophore of *Phyllodoce*, cholinesterase activity has been demonstrated histochemically along the inner surfaces of the ectoderm cells, especially those of the prototroch, by Dr. T. Lacalli, University of British Columbia (personal communication). The veliger synapses structurally resemble interneural synapses in terrestrial gastropods (Amoroso *et al.*, 1964), having a dense population of small synaptic vesicles. All the evidence points to these junctions as the propable mediators of ciliary arrest, but the chemical transmitter concerned is not known. Acetylcholine occurs in gastropod nervous systems (Kerkut and Cottrell, 1963) and is suspected to be the interneural transmitter at junctions having this type of morphology in bivalves (Myers, 1974), but proof is lacking. Buznikov and Manukhin (1962 and earlier reports cited) isolated a substance from veliger larvae which inhibits ciliary beating and decreases sensitivity to the excitatory action of serotonin. They suggest that this substance may be the normal mediator of ciliary arrests. However, it is not acetylcholine, and despite intensive investigation its identity remains unknown. A further argument against acetylcholine as the transmitter comes from Carter's (1926) observation that curare has little or no effect on veligers.

Our electron microscope work has demonstrated the existence of a certain number of velar neurites stuffed wtih dense-cored vesicles. Similar vesicles occur in many other molluscs (Gerschenfeld, 1973). In gastropods, a variety of evidence implicates them as a site of serotonin (Taxi and Gautron, 1969; Cottrell and Osborne, 1970; Jourdan and Nicaise, 1970). In bivalves, on the other hand, they are more likely to contain dopamine (Myers, 1974 and authors cited).

It is well established that serotonin (5-hydroxytryptamine) stimulates the beating of velar cilia in opisthobranch veligers (Koshtoyants *et al.*, 1961; Buznikov and Manukhin, 1962; Korobtsov and Sakharov, 1971) as it does in isolated bivalve gill preparations (Aiello, 1957, 1960; Gosselin, 1961). Jørgenson (1975), who reviews more recent work in this field, found that serotonin did not affect the rate at which intact mussels cleared suspensions of yeast cells. He suggests that the serotoninergic innervation serves only to *maintain* (rather than to accelerate) the activity of the lateral cilia in the intact animal. The mechanism of action of serotonin is uncertain, but it is still effective on veligers when all ions other than magnesium are removed from the external medium (Korobtsov and Sakharov, 1971) which makes it unlikely that the drug acts by changing membrane permeability according to these authors. Paparo and Murphy (1975a and b) give evidence that it works by mobilizing calcium stored intracellularly.

Thus evidence from several sources points to a dual ciliary control mechanism in veligers. Both involve the nervous system, but only one (the arrest system) is likely to be associated with neurociliary synaptic transmission. The other perhaps works by releasing serotonin in tissue spaces near the ciliated epithelium.

It is too early to say how closely this picture corresponds to the situation in bivalves. The apparent absence of neurociliary synapses in bivalves is puzzling since the arrest response is so similar to what we see in veligers. In both cases arrest appears to be dependent on external calcium ions (Korobtsov and Sakharov, 1971; Satir, 1975), and indeed an influx of Ca^{2+} is evidently the critical event in ciliary arrest or reversal generally (see reviews by Naitoh and Eckert, 1974; Aiello, 1974).

Our electrical recordings show that in the gymnosome larvae, and to all appearances in veligers, ciliary arrest is associated with a depolarization lasting some 400 msec. These events (Table I) are much larger than comparable events recently recorded in *Mytilus* by Murakami and Takahashi (1975) and more closely resemble ciliary arrest potentials in tunicates (Mackie *et al.,* 1974). We envisage the depolarizations being initiated by inward synaptic currents and being accompanied by a temporary increase in calcium conductance. Thus they would be excitatory rather than inhibitory events in the usual neurophysiological sense, although the effector response is spoken of in terms of inhibition. Repetitive firing results in sustained depolarization and prolonged arrest. We would like to correlate the electrical and mechanical events by the precise technique exploited by Murakami and Takahashi (1975).

Until more is known about the life of these larvae in the sea, not much can be said about the functional utility of ciliary control, although it seems obvious that any animal which relies so heavily on ciliary effectors would find numerous advantages in being able to regulate their activity. It is reasonable to suppose that ciliary arrests, along with the muscular retraction of the velum which often accompanies them, would serve as a means of protection and escape from damaging stimuli (Fretter, 1967). The veligers of *Nassarius obsoletus* stop swimming and settle on the bottom in response to a chemical factor emanating from suitable substrates (Scheltema, 1961), but it is not clear if this response involves ciliary arrests of the kind we are dealing with here.

With regard to "spontaneous" arrests, G. Richter's studies raise some interesting possibilities. This author (Richter, 1973; Richter and Thorson, 1975) notes that swimming veligers normally swim upward, because the distribution of lighter and heavier parts is such as to cause the velum to face upward. Upward locomotion takes place at a velocity of 36–52 m/hr in the laboratory, which would permit in theory (as appears likely in practice) as ascent of 200 m during the diurnal migration. The velocity of sinking in nonswimming larvae was measured in the laboratory at 72 m/hr. However data for the migration cycle in the sea give a descent rate of only 10 m/hr which is taken to indicate that descending larvae sink in fits and starts, presumably by alternately arresting the cilia for brief periods and then letting them beat again. Thus, spontaneous ciliary arrests may serve a functional role in regulating the rate of sinking during downward migration. It would be worth testing the effects of light on the frequency of ciliary beating since light is an important stimulus for many migrating species (Vinogradov, 1970).

The experimental work was carried out during a visit by G. O. Mackie to France under the NRC-CNRS scientific exchange program, and was further assisted by travel and operating funds from NRC. We thank P. Bougis, Director of the Station Zoologique, for providing the necessary facilities. The electron microscopy was done at the University of Victoria on an instrument granted by the National Research Council of Canada.

SUMMARY

1. The locomotory cilia of *Mangelia* and *Pneumoderma* larvae undergo arrests spontaneously and in response to tactile stimulation. These events are often associated with muscular contractions in an overall response thought to be protective in nature.

2. Isolation of the ciliated bands from the central nervous system abolishes the ability for coordinated ciliary arrests and the cilia show continuous metachronal beating.

3. Recordings with suction electrodes attached to the surface show patterns of electrical signals during periods of ciliary arrest. Intracellular recordings with glass microelectrodes from single ciliated cells in *Pneumoderma* show rapidly rising, slowly decaying, all or none 50 mV spikes when the cilia undergo arrest. There are no fluctuations in membrane potential during metachronal beating.

4. The existence of a rich motor innervation supplying the ciliated epithelium in *Mangelia* has been established using optical and electron microscopy. The nerve endings appear to derive from neurons whose cell bodies are located in or near the central nervous system. The evidence for a local system of neurons forming a nerve net, as described by some authors, is not supported by the present work.

5. Under the electron microscope, neurociliary synapses have been identified. Each ciliated cell in the preoral band of *Mangelia* probably receives at least one synapse. These junctions presumably mediate the arrest response. Synapses are characterized by small, clear presynaptic vesicles in the range 335–560 Å. Similar junctions are made with muscle cells and with supporting (presumed secretory) cells which lie adjacent to the ciliated cells.

6. Neurites containing dense-cored vesicles (560–835 Å) are found near the ciliated cells, but such vesicles are rarely found at synapses and never predominate in them. Taken in conjunction with findings from other gastropods, this observation appears to complement existing pharmacological evidence for an excitatory role for serotonin in molluscan veligers. Comparisons with the dual system of ciliary control found in lamellibranch gills are suggested.

LITERATURE CITED

AIELLO, E. L., 1957. Influence of the branchial nerve and of 5-hydroxytryptamine on the ciliary activity of *Mytilus* gill. *Biol. Bull.*, **113**: 325.

AIELLO, E. L., 1960. Factors affecting ciliary activity on the gill of the mussel *Mytilus edulis*. *Physiol. Zool.*, **33**: 120–135.

AIELLO, E., 1974. Control of ciliary activity in Metazoa. Pages 353–376 in M. A. Sleigh, Ed., *Cilia and flagella.* Academic Press, London.

AMOROSO, E. C., M. I. BAXTER, A. O. CHIQUOINE, AND R. H. NISBET, 1964. The fine structure of neurones and other elements in the nervous system of the giant African land snail *Archachatina marginata*. *Proc. Roy. Soc. London Ser. B*, **160**: 167–180.

BUZNIKOV, G. A., AND B. N. MANUKHIN, 1962. The effect of serotonin on motor activity of nudibranch embryos. *Zh. Obshch. Biol.*, **21**: 347–352.

CARTER, G. S., 1926. On the nervous control of the velar cilia of the nudibranch veliger. *J. Exp. Biol.*, **4**: 1–26.

CARTER, G. S., 1928. The structure of the cells bearing the velar cilia of the nudibranch veliger. *J. Exp. Biol.*, **6**: 97–109.

COTTRELL, G. A., AND N. N. OSBORNE, 1970. Subcellular localization of serotonin in an identified serotonin-containing neurone. *Nature*, **225**: 470–472.

FOL, H., 1875. Etudes sur le développement des Mollusques. Premier Memoire: Sur le développement des Ptéropodes. *Arch. Zool. Exp. Gen. Notes Rev.*, **4**: 1–214.

FRETTER, V., 1967. The prosobranch veliger. *Proc. Malacol. Soc. London*, **37**: 357–366.

FRETTER, V., 1972. Metamorphic changes in the velar musculature, head and shell of some prosobranch veligers. *J. Mar. Biol. Ass. U. K.*, **52**: 161–177.

GALT, C. P., AND G. O. MACKIE, 1971. Electrical correlates of ciliary reversal in *Oikopleura*. *J. Exp. Biol.*, **55**: 205–212.

GERSCHENFELD, H. M., 1973. Chemical transmission in invertebrate central nervous systems and neuromuscular junctions. *Physiol. Rev.*, **53**: 1–119.

GOSSELIN, R. E., 1961. The cilio-excitatory activity of serotonin. *J. Cell. Comp. Physiol.*, **58**: 17–26.

HOLBOROW, P. L., 1971. The fine structure of the trochophore of *Harmothoë imbricata*. Pages 237–246 in D. J. Crisp, Ed., *Fourth European marine biology symposium.* Cambridge University Press.

JØRGENSON, C. B., 1975. On gill function in the mussel *Mytilus edulis* L. *Ophelia*, **13**: 187–232.

JOURDAN, F., AND G. NICAISE, 1970. Cytochimie ultrastructurale de la serotonine dans le système nerveux central de l'Aplysie. *Proc. 7th Int. Congr. Electron Microscopy Grenoble*, **1970**: 677–678.

KERKUT, G. A., AND G. A. COTTRELL, 1963. Acetylcholine and 5-hydroxytryptamine in the snail brain. *Comp. Biochem. Physiol.*, **8**: 53–63.

KNIGHT-JONES, E. W., 1954. Relations between metachronism and the direction of ciliary beat in Metazoa. *Quart. J. Microsc. Sci.*, **95**: 503–521.

KOROBTSOV, G. N., AND D. A. SAKHAROV, 1971. The role of ionic environment in nervous and hormonal control of ciliary beating in molluscan larvae. *Zh. Evol. Biokhim. Fisiol.*, **7**: 238–240.

KOSHTOYANTS, K. S., G. A. BUZNIKOV, AND B. N. MANUKHIN, 1961. The possible role of 5-hydroxytryptamine in the motor activity of embryos of some marine gastropods. *Comp. Biochem. Physiol.*, **3**: 20–26.

MACKIE, G. O., 1974. Behaviour of a compound ascidian. *Can. J. Zool.*, **52**: 23–27.

MACKIE, G. O., A. N. SPENCER, AND R. STRATHMANN. 1969. Electrical activity associated with ciliary reversal in an echinoderm larva. *Nature*, **223**: 1384–1385.

MACKIE, G. O., D. H. PAUL, C. M. SINGLA, M. A. SLEIGH, AND D. E. WILLIAMS, 1974. Branchial innervation and ciliary control in the ascidian *Corella*. *Proc. Roy. Soc. London Ser. B*, 187: 1–35.

MCGEE-RUSSELL, S. M., 1968. The method of combined observations with light and electron microscopes applied to the study of histochemical colourations in nerve cells and oocytes. Pages 183–207 in S. M. McGee-Russell and K. F. Ross, Eds., *Cell structure and its interpretation.* Arnold, London.

MOTOKAWA, T., AND P. SATIR, 1975. Laser-induced spreading arrest of *Mytilus* gill cilia.

J. Cell Biol., **66**: 377–391.

Murakami, A., and K. Takahashi, 1975. Nervous control of cilia: electrical and mechanical responses correlated. *Nature*, **257**: 48–49.

Myers, P. R., 1974. Dopamine: localization of uptake in the pedal ganglion of *Quadrula pustulosa* (Pelecypoda). *Tissue and Cell*, **6**: 49–64.

Naitoh, Y., and R. Eckert, 1974. The control of ciliary activity in Protozoa. Pages 305–352 in M. A. Sleigh, Ed., *Cilia and flagella.* Academic Press, London.

Paparo, A., 1972. Innervation of the lateral cilia in the mussel, *Mytilus edulis* L. *Biol. Bull.*, **143**: 592–604.

Paparo, A. A., and J. A. Murphy, 1975a. The effect of Ca on the rate of beating of lateral cilia in *Mytilus edulis.* I. A response to perfusion with 5-HT, DA, BOL and PBZ. *Comp. Biochem. Physiol.*, **50**: 9–14.

Paparo, A. A., and J. A. Murphy, 1975b. The effect of Ca on the rate of beating of lateral cilia in *Mytilus edulis.* II. A response to electrical stimulation of the branchial nerve. *Comp. Biochem. Physiol.*, **50**: 15–19.

Richter, G., 1973. Field and laboratory observations on the diurnal vertical migration of marine gastropod larvae. *Netherlands J. Sea Res.*, **7**: 126–134.

Richter, G., and G. Thorson, 1975. Pelagische Prosobranchier-Larven des Golfes von Neapel. *Ophelia*, **13**: 109–185.

Satir, P., 1975. Ionophore-mediated calcium entry induces mussel gill ciliary arrest. *Science*, **190**: 586–588.

Scheltema, R. S., 1961. Metamorphosis of the veliger larva of *Nassarius obsoletus* (Gastropoda) in response to bottom sediment. *Biol. Bull.*, **120**: 92–109.

Taxi, J., and J. Gautron, 1969. Données cytochimiques en faveur de l'existence de fibres nerveuses serotoninergiques dans le coeur de l'Aplysie, *Aplysia californica. J. Microsc.*, **8**: 627–636.

Thiriot-Quievreux, C., 1969. Caracteristiques morphologiques des véligères plactoniques de Gasteropodes de la région de Banyuls-sur-Mer. *Vie Milieu*, **20**: 333–366.

Thompson, T. E., 1959. Feeding in Nudibranch larvae. *J. Mar. Biol. Ass. U. K.*, **38**: 239–248.

Vinogradov, M. E., 1970. Vertical distribution of the oceanic zooplankton, *Israel Program for Scientific Translations, Jerusalem.* (Originally published as Vertikal'noe Raspredelnie Okeanicheskogo Zooplanktona. Moskow, 1968.)

Werner, B., 1955. Uber die Anatomie, die Entwicklung und Biologie des Veligers und der Velichoncha von *Crepidula fornicata* L. (Gastropoda, Prosobranchia). *Helgolaender Wiss. Meeresunters. N. F.*, **5**: 169–217.

Reference: *Biol. Bull.,* **151**: 200–213. (August, 1976)

INHERITANCE OF DEMOGRAPHIC AND PRODUCTION PARAMETERS IN THE MARINE COPEPOD *EURYTEMORA HERDMANI*

IAN A. McLAREN

Department of Biology, Dalhousie University, Halifax, Nova Scotia B3H 4J1, Canada

The traits referred to in this report—body size, age of maturity, survivorship, and sex ratio—are important in demography and production of copepods. Biometrical techniques of quantitative or variance genetics are used to estimate the variance among individuals attributable to genetic and environmental sources. The book by Falconer (1960) remains an excellent introduction. Of particular interest is the heritability (h^2) of a trait, which is the ratio of the additive genetic variance to the total phenotypic variance in a population in defined circumstances. Estimation of h^2 allows one, in principle, to predict the response of a trait to selection, at least over a few generations. The techniques of quantitative genetics have been developed largely for use in animal and plant breeding for agriculture and commerce. Studies of the ecological genetics of natural populations have traditionally depended on distinguishable polymorphisms. No mention is made of quantitative genetics in a recent review of the mechanisms of evolution and population genetics of marine organisms (Gooch, 1975), although there are a handful of recent studies (*e.g.,* Chapman, 1974; Doyle, 1974).

The few genetic studies of copepods have been directed at Mendelian traits showing variation within populations, or to differences between local populations in certain quantitative traits (*e.g.*, Battaglia, 1970). Copepods are particularly suitable for applications of quantitative genetics to variation within populations. Earlier work shows that sizes, fecundities, and development rates of a number of copepods are predictable functions of temperature when they are adequately fed (Corkett and McLaren, 1969, 1970). Thus, experimental (environmental) variance can be minimized by controlling temperatures and proffering excess food. The results of such experiments can also be readily translated into demographic models of natural populations (McLaren, 1974).

The copepod *Eurytemora herdmani* Thompson and Scott 1897 is a widespread neritic species. In the western North Atlantic it occurs between northern Labrador (Carter, 1965) and Chesapeake Bay (Heron, 1964). Around Halifax it is common throughout the year and is at times the most abundant copepod in the upper few meters.

MATERIALS AND METHODS

The biometrical analyses of quantitative genetics require rearing of individuals or groups of individuals of known parentage in environments that are as uniform as possible or that differ randomly with respect to the individuals or groups. For the present experiments, female stage V copepodites were removed from fresh plankton samples and given excess food until they matured. Adult males were placed, one to a bottle, in a series of 125 ml bottles and several unfertilized females placed with each of them. Fertile egg sacs usually appeared within hours on some of these females. The females were then isolated in bottles at the temperature at which their offspring were to be reared, and their newly hatched nauplii were removed with fine transfer pipets and placed in labelled vials or bottles.

The keeping of copepods in the laboratory has become commonplace, but the rearing of large numbers of separated individuals is highly laborious. These individuals or small groups were kept in vials or bottles in temperature-controlled rooms, randomly disposed under a variable cycle of diffuse light (usually about 15D : 9L). The rearing medium was membrane-filtered, autoclaved, natural sea water, enriched according to the prescription of Lewis (1967). The containers were swirled usually about three times daily. The animals were removed by fine pipets regularly and their medium and food changed. Sometimes they had to be disentangled from detritus with the aid of fine needles. The food organisms were the flagellate *Isochrysis galbana* and the diatom *Thalassiosira* sp., both reared axenically on half-strength "*f*" medium (Guillard and Ryther, 1962). Each of the three experiments analyzed here differed in ways that are detailed below.

The first experiment (hereafter the 15° C experiment) was begun with 14 males and 28 females captured as copepodites in early May, 1974. Each male fertilized two females and their nauplii all hatched 1000–1900 hr on May 11. Forty-four nauplii from each female were placed, four to a vial, in 25 ml vials. Rearing temperatures, monitored twice daily, ranged from 14.6–15° C (mean 14.9 ± 0.02). The young were fed 75 ml each of six-day-old cultures of *Isochrysis* and *Thalassiosira* made up to one liter with rearing medium. This was changed every fifth day. The diatom did not stay in suspension well and was not used in later experiments. *Isochrysis* flourished in the rearing medium, which remained noticeably green at all times. A few haemocytometer counts during the experiment indicated concentrations of about 1 to 3×10^5 cells per ml, probably far in excess of requirements for maximal growth rates (Corkett and McLaren, 1970). The first offspring matured on May 25, and thereafter the vials were examined in random order every eight hours, a procedure that took some hours while most remained immature.

The second experiment (hereafter the 10° C experiment) was begun with 15 males, each mated to 2 females, both sexes having been reared from wild-caught stage V copepodites. Their nauplii hatched 1000–1700 hr on June 17, 1974. Twenty from each female were reared at 10.0 ± 0.03° C in individual 15 ml vials, which were changed to 25 ml when they began to molt into copepodites. They were fed 150 ml of six-day-old *Isochrysis* made up to one liter with rearing medium, changed every fifth day. The offspring began to mature on July 8 and were checked three times daily thereafter.

The third experiment (hereafter the 12.5° C experiment) was designed for analysis of regressions of offspring sizes on parent sizes. The female parents were chosen to obtain extreme size differences from among large numbers reared from stage V copepodites in each August, 1975. The males were chosen as adults from a large sample from nature. Roughly equal numbers of matings of small × small, medium × medium, large × large, and small × large (for both sexes) were attempted. Offspring from successful matings were fed every fourth day with 225 ml of five-day-old *Isochrysis* made up to one liter with rearing medium. At 12.6 ± 0.02° C, they began to mature on August 9 and were examined daily thereafter.

Ages of maturity were determined to the nearest 0.3 day for the 10° C and 15° C experiments. Offspring were measured at 50× with an optical micrometer, estimating to the nearest 2 μ. Cephalothorax lengths were measured along the dorsal mid-line of the cephalothorax.

A widely used experimental design to estimate heritability (h^2) of a trait in offspring involves mating each male parent (sire) to more than one female (dam). The resulting measurements from offspring are treated by one-way, multilevel, hierarchical (nested) ANOVA, from which components of variance can be determined for each level: between sires, between dams within sires, between replicates (where offspring are reared in within-family groups), and within families. Since it is not possible to prevent differences in mortality, corrections must be made for unequal class sizes. The calculations are fully described by Sokal and Rohlf (1969), whose FORTRAN program was used.

Variance components can be summed and the component for each level expressed as a percentage of this total. This percentage is the intraclass correlation coefficient for the level. The intraclass correlation coefficient between sires is of particular interest as it is a measure of the h^2 of the particular trait that is uninfluenced by possible maternal or common-environment effects. The comparison is between families with the same sire but with different dams; that is, between half sibs with one-fourth of their genetic material in common. So the intraclass correlation coefficient is multiplied by four to give the estimated h^2. The same reasoning applied to the variance component between dams within sires gives an estimate of h^2 that includes any maternal effects. If the dam component is not significantly larger than the sire component, the two may be combined to give a more reliable estimate of the h^2, based on additive genetic effects alone.

Falconer (1963) gives formulas for calculation of symmetrical standard errors of h^2. Since intraclass correlation coefficients are based on variances, confidence intervals should be calculated from F-tables and are asymmetrical. Techniques for calculating such intervals from ANOVA with unequal class sizes are given by Haggard (1958), but this leads to awkwardness. Negative variance components and lower 95% confidence limits are readily ascribable to sampling error, and are traditionally expressed as zero. However, upper confidence limits in excess of 1.0 (the theoretical maximum) were found for a number of estimates of h^2 in this study. For these reasons, confidence intervals for h^2 estimated from ANOVA of sib values are not presented. Rather, probabilities that the estimates differ from zero based on the associated F-tests are given, although this *a posteriori* setting of probabilities can be faulted also.

ANOVA of sib values is an inefficient (although sometimes the only) method of estimating h^2. An alternate estimation is from the regression of the mean value of the trait among sibs against its value in parents. Ordinarily h^2 is taken as equal to the regression coefficient (b) of offspring values on their mid-parent values, or as twice the b of offspring on single parents (each contributing half the genetic material to offspring). Since number of offspring within families varies, the mean family values must be weighted according to the procedure of Kempthorne and

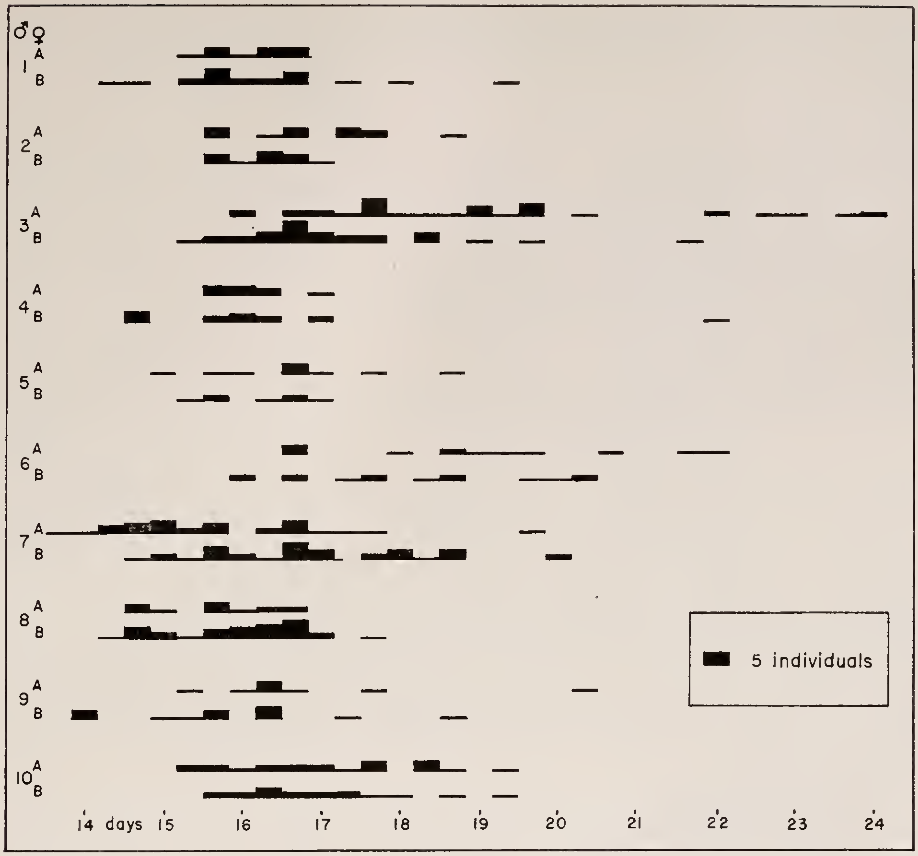


FIGURE 1. Freqeuncy distributions of ages of maturity in female offspring of *E. herdmani* at 15° C. Only families with more than six offspring are included in the figure.

Tandon (1953), as modified by Falconer (1963). This corrects for slope-dependent variance and for the intraclass correlation of families. Falconer's "reasonable estimate" of s.e.$_b$ makes use of the weighted sums of squares and sums of weights as number of degrees of freedom and is used here. Other statistical matters are considered below in context.

RESULTS

Age at maturity

Average times from hatching to maturity were about 28 days at 10° C and about 16 days at 15° C, the females taking longer. These are slightly shorter than the "generation times" (not clearly defined) for this species in the laboratory as given by Katona (1970).

The kinds of data analyzed are shown in Figure 1, on which it is evident that there is a great deal of variation among individuals. Yet, parental effects are

TABLE I

ANOVA and heritability estimates of time to maturity of offspring of E. herdmani.

Temperature	Sex of offspring	Source of variance	Mean square	Per cent of total variance	d.f.	h^2	P
10° C	Male	Between sires	41.60	2.6	14	0.10	0.45
		Between dams, within sires	32.81 (35.03)*	10.4	14 (11.5)	0.42	0.06
		Within families	20.41	87.0	133	—	—
	Female	Between sires	23.28	4.9	14	0.20	0.30
		Between dams, within sires	16.67 (16.60)	0.3	15 (8.7)	(0)	0.50
		Within families	16.88	95.4	85	—	—
15° C	Male	Between sires	12.43	−3.7	13	(0)	0.68
		Between dams, within sires	12.26 (14.72)	28.3	14 (13.2)	1.13	<0.0001
		Between replicates	2.26 (2.26)	16.9	203 (200.9)	—	<0.0001
		Within replicates	1.45	58.5	219	—	—
	Female	Between sires	24.82	17.7	13	0.71	0.05
		Between dams, within sires	8.24 (9.96)	17.7	15 (13.5)	0.72	<0.001
		Between replicates	2.00 (2.02)	8.8	185 (174.9)	—	<0.001
		Within replicates	1.53	55.9	210	—	—

* Terms in parentheses adjusted for unequal class sizes.

also clear; the effects of the male parent are seen, for example, by comparison of the families of male 6 and male 8, and the contribution of females is evident in the two families of male 7.

The 15° C experiment involved up to four surviving animals in each replicate vial, and the component of variance attributable to differences between vials within families is relatively low (Table I). The differences within replicates do, however, include environmental effects to the extent that "accident" plays a role in maturation rate. A distressing feature of these large-scale rearings are a few "stragglers" that matured some time after the others. The delays were not nearly as extreme as those found among species of harpactacoid copepods by Coull and Dudley (1976), who believe that the spread in times within families is adaptive. Probably stragglers of *E. herdmani* had been subjected to some stress or disease during rearing; some were known to have spent time entangled in detritus. There is no obvious way of transforming distributions when only some are skewed this way, although the variances may be inhomogeneous and formally inappropriate for ANOVA. Transformation to ranks might help, but rank ANOVA does not seem to have been fully explored for multilevel cases with unequal class sizes. For present purposes, the effect of "stragglers" is to reduce intraclass correlation coefficients and therefore estimates of h^2.

The ANOVA (Table I) indicates that age of maturity was strongly and significantly heritable only for female offspring in the 15° C experiment. The very similar between-sire and between-dam components suggest that there was no additional maternal contribution in this experiment. In the 10° C experiment female age of maturity was not significantly heritable, nor was there any detectable maternal effect. The results for male offspring are to a degree consistent between the two experiments: age of maturity was not significantly heritable, but there were powerful maternal effects.

Adult size

The ANOVA (Table II) indicates that size was strongly heritable only among males in the 15° C experiment. The between-dam, within-sire components for both male and female offspring were similar to the between-sire components in the 15° C experiment. This suggests that there were no maternal effects and that the between-sire estimate of h^2 in size of female offspring, although not itself significant, is real. In the 10° C experiment there were low, nonsignificant estimates of h^2 and strong maternal effects.

Estimates of h^2 in size were also derived from regressions (*e.g.*, Fig. 2). Some of the parents in the 10° C and 15° C experiments died and disintegrated before they could be measured.

In using mid-parent values or the sib and dam values separately, it has to be assumed that the population variance of the trait are the same in males and females. However, dams of *E. herdmani* were distinctly more variable in length than were sires (F, 2.50; d.f., 25, 14, at 10° C; F, 7.01; d.f., 12, 7, at 15° C). The same was true for offspring (F, 2.14; d.f., 103, 140, at 10° C; F, 1.29; d.f., 181, 222, at 15° C). In view of these disparities, it was necessary to equalize standard deviations (using average s.d. of the two sexes) for parents and offspring, respectively, within each experiment. This ensured that each parent contributed comparably to the

TABLE II

ANOVA and heritability estimates of adult size of offspring of E. herdmani.

Temperature	Sex of offspring	Source of variance	Mean square	Per cent of total variance	d.f.	h^2	*P*
10° C	Male	Between sires	7.54	−0.7	14	(0)	0.52
		Between dams, within sires	7.11 (7.87)*	18.7	14 (11.9)	0.75	0.01
		Within families	3.32	82.0	132	—	—
	Female	Between sires	13.12	6.0	14	0.24	0.35
		Between dams, within sires	8.96 (10.36)	23.3	15 (11.9)	0.93	0.02
		Within families	4.29	70.0	85	—	—
15° C	Male	Between sires	33.59	24.2	13	0.97	0.04
		Between dams, within sires	9.69 (11.60)	18.9	14 (13.1)	0.76	0.001
		Between replicates	2.04 (2.05)	15.5	202 (199.6)	—	0.007
		Within replicates	11.84	41.3	215	—	—
	Female	Between sires	22.41	9.6	13	0.38	0.14
		Between dams, within sires	10.42 (11.60)	12.0	13 (11.9)	0.48	<0.001
		Between replicates	3.67 (3.68)	15.4	188 (185.9)	—	<0.001
		Within replicates	2.49	63.0	205	—	—

* Terms in parentheses adjusted for unequal class sizes.

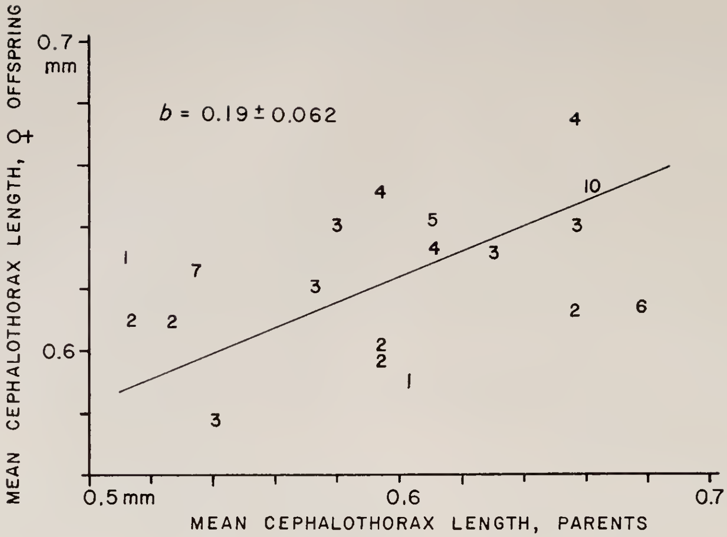


FIGURE 2. The relationship between mean parent size and mean size of female offspring at 12.5° C. The numbers are family sizes used in calculating weights for means used in the regression.

mid-parent value and that *b* for different parent-offspring regressions was not sex-dependent.

The results of regression analysis (Table III) reinforce conclusions from ANOVA (*cf.*, Table II). Heritabilities were high at 15° C and low, although still significant from midparent regressions, at 10° C. The indication of maternal effects in ANOVA may be reflected in the high values of h^2 from regressions on dams; it is

TABLE III

Estimates of heritabilities of adult size of offspring of E. herdmani *based on parent-offspring regressions*

Temperature	Regression	d.f.*	b	h^2	s.e. h^2
10° C	Male offspring vs. mean parent	87.1	0.180	0.18	0.104
	Male offspring vs. male parent	86.1	−0.180	−0.36†	0.181
	Male offspring vs. female parent	92.4	0.233	0.47	0.151
	Female offspring vs. mean parent	64.3	0.166	0.17	0.061
	Female offspring vs. male parent	75.2	−0.316	−0.63†	0.209
	Female offspring vs. female parent	84.7	0.444	0.89	0.161
12.5° C	Male offspring vs. mean parent	52.7	0.185	0.19	0.046
	Female offspring vs. mean parent	39.5	0.191	0.19	0.062
15° C	Male offspring vs. mean parent	33.8	0.608	0.61	0.118
	Male offspring vs. male parent	48.3	0.368	0.74	0.107
	Male offspring vs. female parent	22.0	0.194	0.39	0.378
	Female offspring vs. mean parent	65.8	0.514	0.51	0.153
	Female offspring vs. male parent	68.1	0.300	0.60	0.149
	Female offspring vs. female parent	45.9	0.203	0.41	0.399

* Sum of weights of the means used in the regressions (see text).

† Negative values of h^2 are not theoretically possible.

as though the effect were mediated through female size. The accompanying negative values for sires are inexplicable.

Since some parents for the 12.5° C experiment were chosen for extreme sizes, population variances cannot properly be estimated. Furthermore, their male and female offspring turned out to be similar in length variation (F, 1.06; d.f. 65, 102). Therefore no attempt was made to equalize standard deviations for estimates of b and h^2. The mid-parent values of h^2 for size at 12.5° C are low, but with substantially reduced s.e. compared with those at 10° C. Of course single-parent estimates of b in the 12.5° C experiment would be artificially biased upward by correlation of parent sizes and were not used to calculate h^2.

Parental selection or assortative mating itself should cause only negligible bias in regression estimates as long as h^2 is low (Hill, 1970). However, there could have been another source of bias in the 12.5° C experiment. The dams were matured from stage V copepodites, which were probably a near-cohort that had experienced a common environment. The sires, however, were taken as adults in nature in order to maximize the available size range. They may have matured over a period of time in a variety of conditions. Increased environmental variance in their sizes would have the effect of depressing estimates of b for offspring on sires as compared with dams. However, there is no evidence that this happened. Estimates of $b \pm \text{s.e.}_b$ for offspring on single parents were: 0.11 ± 0.042 for males on males; 0.20 ± 0.045 for males on females; 0.16 ± 0.051 for females on males; and 0.15 ± 0.063 for females on females. (The implied, but invalid, estimates of h^2 are larger than those from mid-parent regressions for reasons noted above.) It is concluded that, in spite of differences in experimental design, the results at 12.5° C are comparable with those from the other two experiments.

Correlation of size and age of maturity

Increase in temperature decreases development time and normally decreases size of copepods (Deevey, 1960; McLaren, 1974). However, as Katona (1970) had already shown, temperature may not influence size of *E. herdmanii*; means and s.e. were $671 \pm 7\ \mu$ and $667 \pm 5\ \mu$ for males and $769 \pm 6\ \mu$ and $758 \pm 15\ \mu$ for females at 10° C and 15° C, respectively. Furthermore, size and age of maturity were not inversely related within experiments. This is adequately demonstrated without analysis of covariance with two simple nonparametric tests.

The rank correlations between family medians of sizes and ages of maturity (Table IV) were all negative and highly significant except for female offspring at 15° C. Within families, the individuals born before the median age of maturity

TABLE IV

Spearman rank correlation of median cephalothorax lengths and median times to maturity among families of E. herdmani.

Temperature	Sex	r_s	d.f.	P
10° C	Male	−0.61	24	0.01
	Female	−0.66	20	0.01
15° C	Male	−0.53	23	0.01
	Female	−0.15	23	0.48

TABLE V

ANOVA for binomial data of mortality of E. herdmani *before maturity. Estimates of* h² *made after probit transformation (see text).*

Temperature	Source of variance	Mean square	Per cent of total variance	d.f.	h^2	s.e. h^2
10° C	Between sires	0.384	0.3	14	0.01	0.107
	Between dams, within sires	0.352	2.3	15	0.08	0.111
	Within families	0.244	97.5	570	—	—
15° C	Between sires	0.643	0.4	13	0.02	0.043
	Between dams, within sires	0.575	4.2	14	0.13	0.101
	Within families	0.196	95.4	1204	—	—

were more likely to be larger than median size. At 15° C the numbers were 103:57 for males and 86:57 for females. At 10° C the numbers were 53:14 and 31:6, respectively. Again only for females at 15° C is this not significant (by Chi², corrected for continuity).

It thus appears that small adults on average took longer to mature. This implies that heritabilities of growth rates (size/time) would be higher than those for sizes and ages of maturity separately.

Mortality

Mortality was 52% at 10° C and 29% at 15° C; but since maturation time averaged longer at 10° C, instantaneous death rates were quite similar: 0.025 at 10° C and 0.021 at 15° C on a daily basis. Mortality was quite variable between families, and it is of some interest to calculate heritabilities. Mortality is not a continuous trait in individuals, but can be thought of as a threshold phenomenon expressed when the sum of independent, normally distributed environmental and genetic components exceeds a certain "dose." Intraclass correlation coefficients can be estimated from ANOVA for binomial data (Lush, Lamoreux and Hazel, 1948). The additive heritabilities on the observed probability scale can then be calculated after probit transformation (Dempster and Lerner, 1950 and references therein).

Although the ANOVA (Table V) indicates a highly significant between-dam, within-sire component at 15° C (F, 2.94; d.f. 14, 1204), the heritability estimates are small and do not differ from zero, according to their standard errors. These symmetrical s.e., calculated as suggested by Dempster and Lerner (1950), are only approximate. The above F-test and the fact that the between-dam estimates of h^2 are substantially higher than the between-sire ones at both 10°C and 15° C suggest that there is a real maternal effect on survivorship.

Sex ratio

Overall sex ratios (male/total) were 162/281 (58%) at 10° C and 450/827 (51%) at 15° C. These ratios do not differ significantly from one another (Chi², corrected for continuity, 3.18; *P*, 0.07).

Individual families varied markedly, some being all males and others all females. Six ratio can be treated as a threshold trait using the analyses applied above to mortality. In this case, the unequal class sizes called for use of adjusted d.f. in calculating F ratios and s.e. (Table VI). Both sire and dam appeared to influence sex ratios of their families equally at both temperatures, so that intraclass correlation coefficients can be combined to give more reliable estimates for h^2 of sex ratio of 0.12 ± 0.13 (adjusted d.f. 18.7) at 10° C and 0.30 ± 0.13 (adjusted d.f. 62.8) at 15° C. A significant effect at 15° C is also implied in the F-ratios (from mean squares on Table VI).

Sex ratios were distinctly bimodal among the families at 15° C and probably at 10° C as well (Fig. 3). This suggests that there is major-gene control of high-male and low-male status of families, with polygenic overlay of variance. Since low-male families occurred in both families of two sires and in only one family of three other sires, the major-gene control appears to be autosomal.

For convenience, within-vial variance was ignored in the ANOVA of sex ratio at 15° C, and this might have inflated the estimated h^2 to the extent that it is environmentally influenced. Although Katona (1970) found no correlation between sex ratio and number of progeny in a tube (no data given) for *Eurytemora* (sp.?), the possibility of more subtle effects was worth checking. These could result, for example, from pheromonic influences so that extreme sex ratios (maximally 0/4 or 4/4) were promoted. Among vials with 4 survivors in 13 families with reasonably balanced ratios (39–72% male), the expected frequencies of extremes (0/4, 4/4) were calculated from binomial expectations, using family ratios as p:q. The observed number of extremes (9) was very close to the expected (10.9).

Given the distorted sex ratios, it is possible that some individuals of one sex tended to have attributes of the other. For example, females average larger in size and slower in maturation than males, and heritable sex ratios could have influenced the h^2 of size and age of maturity within sexes. However, female offspring in low-male families do not differ significantly (randomized test, Siegel, 1956) from those in high-male families in median body size (t, 1.22; d.f., 25) or median age of maturity (t, 1.08; d.f., 25). The medians for male offspring in low-male families are based at most on a few individuals, but also appear to fall within the range for high-male families. It is concluded that offspring are "real" males and females for our purposes.

TABLE VI

ANOVA for binomial data of sex ratio in offspring of E. herdmani. *Estimates of* h^2 *made after probit transformation (see text).*

Temperature	Source of variance	Mean square	Per cent of total variance	d.f.	h^2	s.e. h^2
10° C	Between sires	0.479	3.4	14	0.11	0.218
	Between dams, within sires	0.317	3.9	15	0.13	0.221
	Within families	0.288	92.7	251	—	—
15° C	Between sires	3.454	12.0	13	0.27	0.153
	Between dams, within sires	1.507	16.9	14	0.33	0.171
	Within families	0.180	71.1	849	—	—

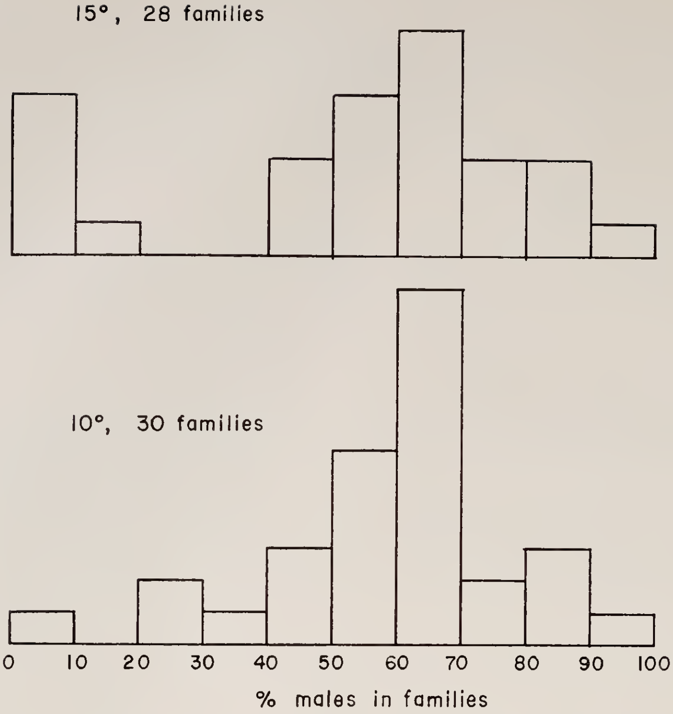


FIGURE 3. Frequency distributions of sex ratios in families of *E. herdmani.*

DISCUSSION

Biological oceanographers have traditional concerns with the effects of salinities, temperatures, and food supplies on marine copepods. In the laboratory, individual variability, if not ignored, is taken as "experimental error." Paffenhöfer (1970) found that differences in size and sex ratios in *Calanus helgolandicus* were correlated with food quantity and quality; but these differences were accompanied by marked variations in mortality, which could have been selective. Furthermore, it is probable that a small number of parents were used to produce the young reared in some of Paffenhöfer's experiments. Certainly the percentage differences between means in some of his experiments are well within the ranges shown by individuals and sometimes family means in the present studies of *Eurytemora herdmani.* This is not a serious criticism of his conclusions, but rather a more general request that precautions be taken (and reported) that minimize effects of inherent differences among experimental copepods.

It is not surprising that genetic differences in quantitative traits are being found between populations of the "same" copepod from different localities (*e.g.*, Carillo, Miller and Weibe, 1974) or even from different seasons (Bradley, 1975). There is a general lack of knowledge concerning the extent of heritable variation *within* populations of copepods and the ecological meaning of this variation in nature. Since this study of *E. herdmani* seems to be the only one of its sort for a planktonic copepod, it is not yet possible to seek clues from comparative studies of the group. However, the result can be compared with more general principles.

Much of the current controversy about genetic variation in nature (Lewontin, 1973) refers to biochemical differences that have no clear effects on the fitness of individuals. It is generally supposed that traits of significance to fitness will have their heritable variation trimmed away by natural selection in the normal environment. Age of maturity and size (which affects female fecundity) are demographic parameters and are clearly components of fitness. Therefore, their generally low heritabilities at 10° C and 12.5° C are not surprising. These temperatures are normal during summer in near-surface waters around Halifax. The much larger heritabilities of size in both sexes and of age of maturity in females in the 15° C experiment are reasonable, since such temperatures rarely occur, even at the surface. Even at this high temperature there are revealing differences between the sexes: heritability of size was lower for females, and age of maturity was not detectably heritable in males. Size is important in determining the egg number of a whole series of clutches in females (Corkett and McLaren, 1969; McLaren, 1974), whereas age of maturity is obviously more important to males, which may fertilize a series of females in short order, but which do not benefit in any obvious way from differences in size.

Although maternal effects are not properly the concern of ecological genetics, they are by no means devoid of interest as adaptive tactics in their own right. In the present experiments there were powerful maternal influences on age of maturity of males, but not females. Conversely, the between-dam component of size was higher for female offspring which, for reasons noted above, would benefit most from this. It is easy to see how the dam could hasten the maturity of her offspring through investments in the egg. Some families of nauplii appeared to have more pigment (lipid?) and to be more vigorous in swimming upon hatching. The stronger between-dam component in morality may also be based largely on differences in early mortality. It is, however, less easy to see how differences in size of adult males could be mediated through a tiny egg.

Unbalanced sex ratios are well known in nature, but difficult to account for in theory (Fisher, 1930; and others since). The discussion by Katona (1970) of the phenomenon in *Eurytemora* in terms of population advantages is naive. Furthermore, the supposed effects shown by him of salinity and temperature will have to be reconsidered in the light of the marked variation among families shown here. The high-male and low-male status of families of *E. herdmani* resembles the occurrence of "sex-ratio," a sex-linked major gene, in *Drosophila* (Policansky, 1974), for which explanations are complicated at best. A careful analysis of genotype-environment interactions in determining sex ratio in *E. herdmani* would probably be revealing.

Finally, although this is the first such study of a copepod, *E. herdmani* should not be taken as "typical." The species around Halifax seems to reproduce more-or-less continuously, with adult males and ovigerous females almost always present, and shows little size variation within samples and through the season. By contrast, the genus *Pseudocalanus* seems to carry eggs sporadically and shows much more size variation in nature. Ongoing studies in this laboratory indicate that there are probably sibling species involved, and that size and age of maturity are much more strongly heritable within populations at natural temperatures.

The work reported here is supported by grants from the National Research Council of Canada. I am grateful to Roger Doyle, Trudy Mackay and Gary Newkirk for biometrical advice, and to Joanne Bishop, Ann Linton, and Judith Robins for laboratory assistance.

SUMMARY

Heritabilities (h^2, the ratio of additive genetic variance to total phenotypic variance) were estimated for a number of traits of *Eurytemora herdmani* from families of known parents reared with excess food at 10°, 12.5°, and 15° C. Age of maturity was strongly heritable only among female offspring at 15° C and size only among male offspring at 15° C. This temperature is extreme for waters near Halifax, Nova Scotia. Low and sometimes nonsignificant heritabilities at 10° and 12.5° C are as expected at these natural temperatures for fitness traits that have had their genetic variance trimmed by natural selection. There were strong maternal (nongenetic) effects on age of maturity of males (which benefit from accelerated maturation) and on size, especially of females (which would benefit from enhanced fecundity). Adult size and age of maturity are negatively correlated, so that growth rate should be even more strongly heritable. There is a suggestion that survivorship (not significantly heritable) is maternally influenced (probably along with development rate) early in life. Unbalanced sex ratios appear to be controlled by a major gene, with polygenic overlay of variance. The genetic variations reported here may be important in the design of experiments with copepods, indicate capacity for quite rapid change of some traits in nature, but should not at this stage be taken as "typical" of marine copepods.

LITERATURE CITED

Battaglia, B., 1970. Cultivation of marine copepods for genetic and evolutionary research. *Helgolaender Wiss. Meeresunters.*, **20**: 385–392.

Bradley, B. P., 1975. The anomalous influence of salinity on temperature tolerance of summer and winter populations of the copepod *Eurytemora affinis*. *Biol. Bull.*, **148**: 26–34.

Carillo, B.-G. E., C. B. Miller, and P. H. Weibe, 1974. Failure of interbreeding between Atlantic and Pacific populations of the marine calanoid copepod *Acartia clausi* Giesbrecht. *Limnol. Oceanogr.*, **19**: 452–458.

Carter, J. H., 1965. The ecology of the calanoid copepod *Pseudocalanus minutus* Krøyerin Terriarsuk, a coastal meromictic lake of northern Labrador. *Limnol. Oceanogr.*, **10**: 345--353.

Chapman, A. R. O., 1974. The genetic basis of morphological differentiation in some *Laminaria* populations. *Mar. Biol.*, **24**: 85–91.

Corkett, C. J., and I. A. McLaren, 1969. Egg production and oil storage by the copepod *Pseudocalanus* in the laboratory. *J. Exp. Mar. Biol. Ecol.*, **3**: 90–105.

Corkett, C. J., and I. A. McLaren, 1970. Relationships between development rate of eggs and older stages of copepods. *J. Mar. Biol. Ass. U.K.*, **50**: 161–168.

Coull, B. C., and E. W. Dudley, 1976. Delayed naupliar development of meiobenthic copepods. *Biol. Bull.*, 150: 38–46.

Deevey, G. B., 1960. Relative effects of temperature and food on seasonal variations in length of marine copepods in some eastern American and western European waters. *Bull. Bingham Oceanogr. Collect. Yale Univ.*, **17**: 54–86.

Dempster, E. A., and I. M. Lerner, 1950. Heritability of threshold characters. *Genetics*, **35**: 212–236.

Doyle, R. W., 1974. Choosing between light and darkness: the ecological genetics of photic behaviour of the planktonic larvae of *Spirorbis borealis*. *Mar. Biol.*, **25**: 311–317.

Falconer, D. S., 1960. *Introduction to quantitative genetics.* Ronald Press, New York, 365 pp.

Falconer, D. S., 1963. Quantitative inheritance. Pages 193–215 in W. J. Burdette, Ed., *Methodology in mammalian genetics.* Holden-Day, San Francisco.

Fisher, R. A., 1930. *The genetic theory of natural selection*, 1958 edition. Dover, New York, 291 pp.

Gooch, J. L., 1975. Mechanisms of evolution and population genetics. Pages 349–409 in O. Kinne, Ed., *Marine Ecology, Vol. II, Part 1, Physiological mechanisms.* Wiley-Interscience, New York.

Guillard, R. R. L., and J. H. Ryther, 1962. Studies on marine planktonic diatoms. 1. *Cyclotella nana* Hustedt and *Detonula confervacea* (Cleve) Gran. *Can. J. Microbiol.*, **8**: 229–239.

Haggard, E. A., 1958. *Intraclass correlation and the analysis of variance.* Dryden Press, New York, 171 pp.

Heron, G. A., 1964. Seven species of *Eurytemora* (Copepoda) from northwestern North America. *Crustaceana*, **7**: 199–211.

Hill, W. C., 1970. Design of experiments to estimate heritability by regression of offspring on selected parents. *Biometrics*, **26**: 566–571.

Katona, S. K., 1970. Growth characteristics of the copepods *Eurytermora affinis* and *E. herdmani* in laboratory cultures. *Helgolaender Wiss. Meeresunters.*, **20**: 373–384.

Kempthorne, O., and O. B. Tandon, 1953. The estimation of heritability by regression of offspring on parents. *Biometrics*, **9**: 90–100.

Lewis, A. G., 1967. An enrichment solution for culturing the early developmental stages of the planktonic marine copepod *Euchaeta japonica* Marukawa. *Limnol. Oceanogr.*, **12**: 147–148.

Lewontin, R. C., 1973. Population genetics. *Ann. Rev. Genetics*, **7**: 1–17.

Lush, J. L., W. F. Lamoreaux, and L. N. Hazel, 1948. The heritability of resistance to death in the fowl. *Poultry Sci.*, **27**: 375–388.

McLaren, I. A., 1974. Demographic strategy of vertical migration by a marine copepod. *Amer. Natur.*, **108**: 91–102.

Paffenhofer, G. A., 1970. Cultivation of *Calanus helgolandicus* under controlled conditions. *Helgolaender Wiss. Meersesunters.*, **20**: 346–359.

Policansky, D., 1974. "Sex ratio," meiotic drive, and group selection in *Drosophila pseudoobscura. Amer. Natur.*, **108**: 75–90.

Siegel, S., 1956. *Nonparametric statistics for the behavioral sciences.* McGraw-Hill, New York, 312 pp.

Sokal, R. J., and F. J. Rohlf, 1969. *Biometry.* Freeman, San Francisco, 776 pp.

Reference: *Biol. Bull.*, **151**: 214–224. (August, 1976)

PODOCORYNE SELENA, A NEW SPECIES OF HYDROID FROM THE GULF OF MEXICO, AND A COMPARISON WITH *HYDRACTINIA ECHINATA*

CLAUDIA E. MILLS[1]

Department of Biological Science, Florida State University, Tallahassee, Florida 32306

Based on the adult medusa, Edwards (1972) has recently described a second species of *Podocoryne* from the New England coast of North America, *Podocoryne americana*, which had previously been called *P. carnea* Sars. *Podocoryne americana* attains an adult size of 3.5 mm in height with 24 to 32 marginal tentacles. *Podocoryne carnea*, which is also found in New England, is smaller and has fewer tentacles. The polyps of *P. carnea* and *P. americana* are very similar and the developmental stages of the medusae may also be confusingly similar, so that mature medusae may be necessary for positive identification of these two species.

In this paper a third species of *Podocoryne* from North America, found on the north Florida coast of the Gulf of Mexico, is described. *Podocoryne selena* n. sp. is probably the same hydrozoan that has been referred to as *P. carnea* in north Florida by McLean (1975), Menzel (1971), Wells (1969), Shier (1965), and Joyce (1961). In spite of repeated attempts, this author has never collected *P. carnea* in Florida. *Podocoryne selena* n. sp. may also be identical to *P. carnea* in Texas (Wright, 1973; Defenbaugh and Hopkins, 1973; and Deevey 1950, 1954) and Louisiana (Cary and Spaulding, 1909). The polyp of *P. selena* n. sp. is very similar to the polyp of *P. carnea*; medusae must be examined for positive identification in other coastal areas in order to establish the geographic ranges of the species of *Podocoryne* in North America.

The hydroids *Podocoryne* and *Hydractinia* live on hermit crab shells and other hard substrates on the Atlantic and Gulf coasts of the United States. In New England specimens of *Podocoryne carnea* and *Hydractinia echinata* are found in the same areas (Crowell, 1945), as are *P. selena* n. sp. and *H. echinata* in north Florida. For the non-specialist, it is sometimes difficult to distinguish *Podocoryne* from *Hydractinia*, both of which belong to the family Hydractiniidae. *Podocoryne selena* n. sp. and *H. echinata* are compared in this paper through the use of both phase contrast and scanning electron microscopy.

MATERIALS AND METHODS

Hydroid colonies of *Podocoryne selena*, n. sp. were collected on the shells of live hermit crabs in the following locations in Frankin County, Florida: Alligator Point; Alligator Harbor; Baymouth Bar near Alligator Point and at the opposite end of the bar near St. Teresa; Wilson's Beach at St. Teresa; Turkey Point; and in St. Joseph Bay in Gulf County at several points between Presnell's Fish Camp and

---

[1] Present address: 7044 50th Ave. N. E., Seattle, Washington, 98115.

Black's Island. Animals were collected on sandy bottoms either by wading or snorkeling in 0 to 3 meters of water.

The hermit crabs and hydroid colonies were kept in the laboratory in 20-gallon glass aquaria containing natural sea water and were fed pieces of fish, shrimp, or octopus, and *Artemia* nauplii. The water was maintained at room temperature, approximately 20° C, and was filtered with both a sub-gravel filter and a Dyna-Flow outside filter.

Podocoryne selena n. sp. medusae were obtained by placing a shell covered with a colony of *Podocoryne* polyps with medusa buds in a bowl of sea water and exposing them to either bright daylight or artificial light. Medusae began to be liberated after about fifteen minutes and continued to be released for several hours. The newly released medusae were transferred to a fingerbowl of clean sea water with a pipette and were fed *Artemia* nauplii which they were able to ingest whole. The water was aerated gently. The medusae were transferred by pipette to a clean bowl of sea water every one or two days. Enough *Artemia* nauplii were added to sustain the medusae until the next change of water.

Nematocysts were identified using the key of Mariscal (1974a). Measurements of undischarged nematocysts were made using a Reichert phase-contrast microscope. Size ranges were determined by measuring at least ten of each type of nematocyst.

Specimens were prepared for the scanning electron microscope (SEM) by the procedure described by Mariscal (1974b) with the following modifications: prior to immersion in 16% glycerol for 24 hours, specimens were fixed in Parducz (1967) solution of six parts 2% aqueous OsO_4 and one part $HgCl_2$ saturated sea water for 45 minutes. Photographs were taken on Polaroid 105 Positive Negative Film by William Miller on a Cambridge Stereoscan S4-10 scanning electron microscope.

TAXONOMY

Podocoryne selena new species (Figs. 1–4).

Diagnosis

Female medusae released with 8 marginal tentacles, male medusae released with 5 to 8 marginal tentacles; gonads with nearly mature sexual products at time of release. Tentacle number increases to up to 14 in two weeks in the laboratory. Maximum size reared was 1.8 mm in bell height and diameter. Polyps nearly identical to polyps of *P. carnea*. *Selene* is the ancient Greek name for the moon.

Description

Medusa (*Figs. 1, 2*). Newly released female medusae have 8 marginal tentacles and measure 0.8 mm in bell height and diameter. The bell is nearly spherical and the jelly is thin. The manubrium hangs down about two-thirds the length of the subumbrellar cavity. The mouth has four lips, each consisting of a battery of about 60 individual pendant microbasic euryteles (see Fig. 2). The surfaces of the mouth and manubrium are provided with scattered long, slow-beating cilia, as are the tentacles and inside surfaces of the radial canals. At release, the 4 perradial tentacles have well developed basal bulbs and the 4 interradial tentacles, which are somewhat shorter, have rudiments of bulbs. The tentacles and bulbs are

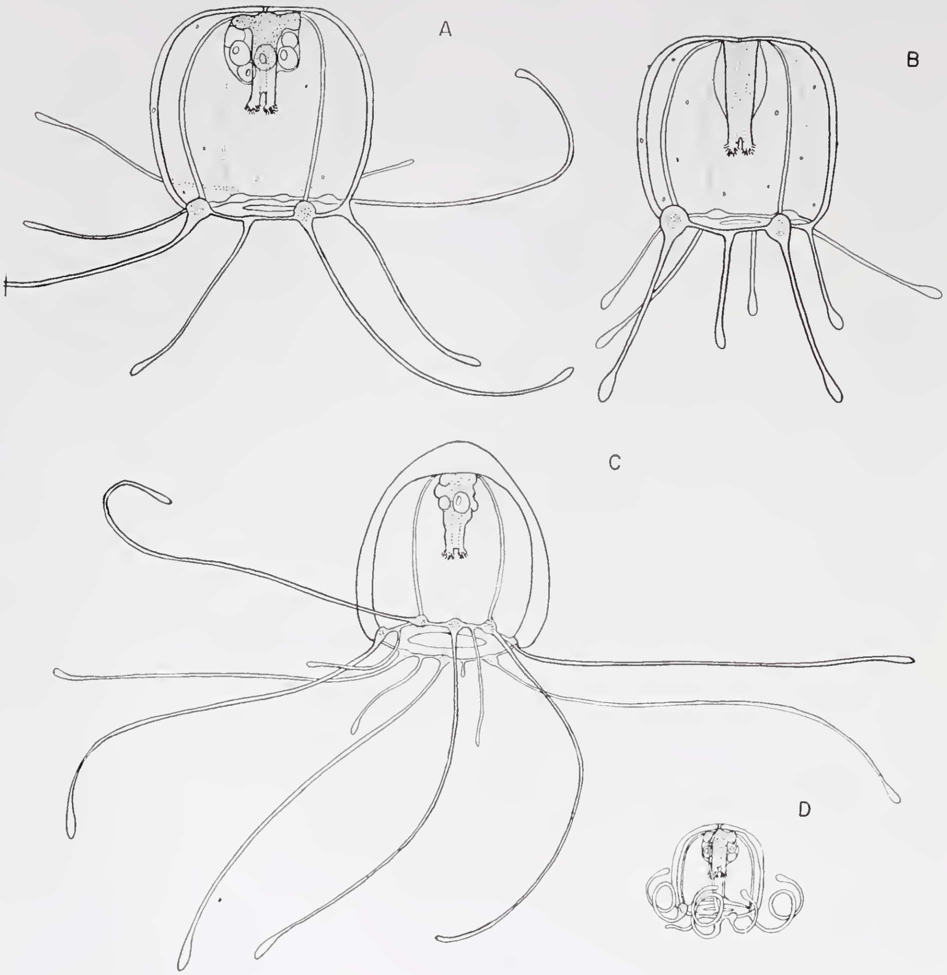


Figure 1. *Podocoryne selena* medusae from hydroid colony on hermit crab shell in north Florida Gulf of Mexico, all drawn from life. A. Newly released female, 0.8 mm in bell height and diameter, with nearly mature eggs in gonad surrounding manubrium. B. Newly released male, 0.6 mm in bell height and diameter, with immature testis surrounding manubrium. C. Female 13 days old, 1.6 mm in bell height and diameter. D. Newly released female, 0.8 mm in bell height and diameter, drawn to scale with mature female of Figure 1C; shown in typical posture with partly contracted tentacles.

sprinkled heavily with desmonemes and microbasic euryteles, not arranged into batteries. There are also a few nematocysts on the margin of the exumbrella between tentacles in the region of the ring canal. The exumbrella is very sparsely provided with desmonemes. The complete cnidom of *P. selena* is given in Table I. The four radial canals and ring canal are narrow. A short umbilical canal is evident on newly released specimens. The velum is broad. Many eggs with germinal vesicles can be seen on female medusae in the gonads surrounding the manubrium. Male medusae measuring 0.6 mm in bell height and diameter are released with 5 to 8 tentacles. The testis appears as a clear swelling around the manubrium which becomes milky white when the sperm mature. Eggs and sperm mature within 2 to 3 days, at which time the release of eggs begins. Eggs were released for 12 days in the laboratory. Sexually mature female medusae measured 1.2 mm in height and diameter when 3 days old. Sexually mature males measured 0.9 mm in height and diameter when 3 days old. Maximum size attained with a 2-week old female medusa measuring 1.8 mm in height and diameter. An incomplete second cycle of tentacles appeared in most specimens during the second week of life under laboratory conditions. Mature medusae were seen with 8 to 14 tentacles in two weeks and were usually 1.0 to 1.5 mm high at that time.

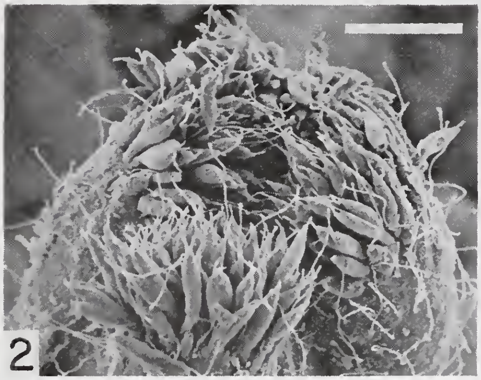
Polyps (*Fig. 3, 4*). The polyps of *Podocoryne selena* are similar to those of *P. carnea* as discussed by Edwards (1972) with differences as noted below. Spines in colonies of *P. selena* are usually up to 1.2 mm high, whereas those of *P. carnea* are reported by Edwards to be 0.2–0.3 mm high. The hydrorhizal base of the colony of *P. carnea* often grows out beyond the aperture of the shell-substrate which effectively enlarges the shell (Edwards, 1972). This type of growth has not been observed in *P. selena* in Florida.

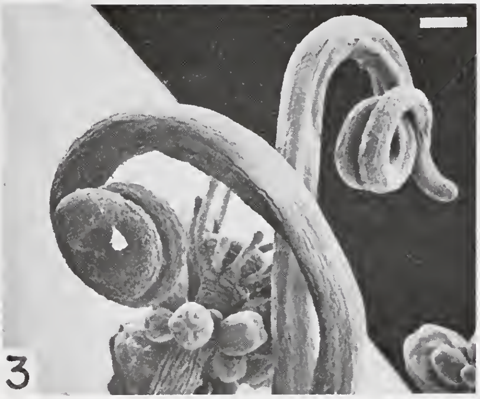
Spiral zooids are usually present in *Podocoryne selena* colonies at the apertures of hermit crab shells and are most often located in a single row only on the shell edge above the crab. They are sometimes found encircling the entire shell aperture and may occur in a band several polyps deep. Spiral zooids were never seen in colonies of *P. selena* that covered shells of the live gastropod *Cantharus cancellarius*.

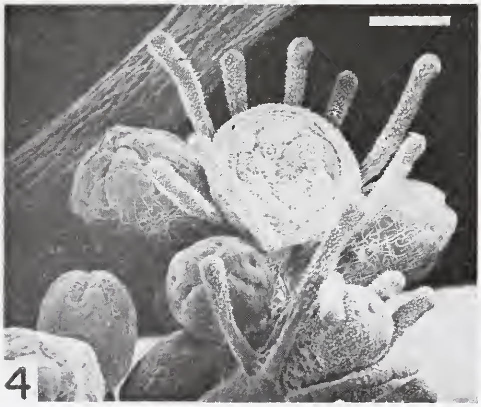
TABLE I

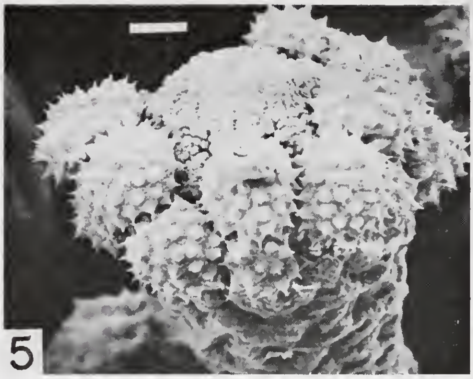
Nematocysts of Podocoryne selena *and* Hydractinia echinata *from the north Florida Gulf of Mexico. All capsules were measured undischarged; measurements are expressed in μm. An asterisk indicates that a nematocyst was present, but not measured.*

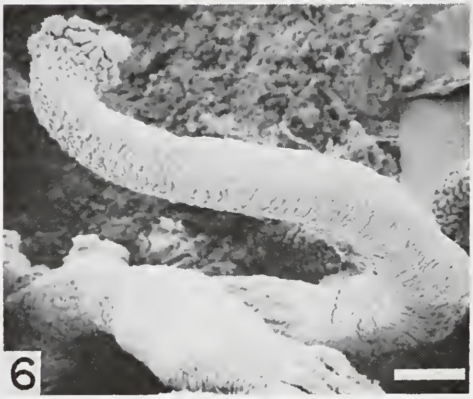
	Podocoryne selena		*Hydractinia echinata*	
	Desmonemes	Microbasic euryteles	Desmonemes	Microbasic euryteles
Hydroid				
Spiralzooid	None	12.0–14.5 × 4.0–5.0	None	12.0–14.0 × 4.0–5.0
Gonozooid	*	*	None	*
Gonophore	None	None	None	8.0–9.0 × 2.0 13.0 × 5.0
Feeding zooid	5.0–6.0 × 3.0	7.0–10.0 × 2.5–3.0 12.0–15.0 × 5.0–7.0	6.0–6.5 × 3.0	9.0–10.0 × 3.0–4.0 12.0–13.0 × 5.0–6.0
Medusa				
Exumbrella	5.0–6.0 × 3.0	None	Hydroid has fixed gonophores, no medusae	
Manubrium	None	9.0–11.5 × 3.0		
Tentacles	5.0–6.0 × 3.0	7.0–8.0 × 3.0		


2


3


4


5


6

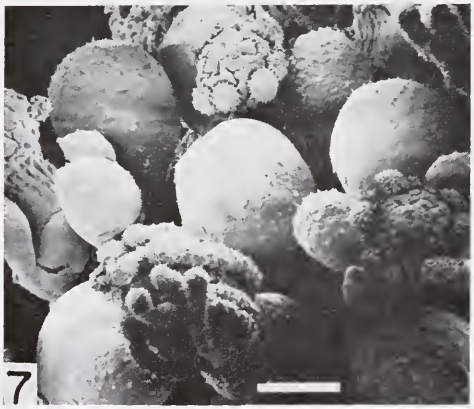

7

FIGURE 2. Mouth of *Podocoryne selena* with four lips, each consisting of a battery of about 60 individual pendant microbasic euryteles. Notes the long, flexible, cilia located on the lips and the surface of the manubrium. Scale bar equals 20 μm.

FIGURE 3. Two spiral zooids of *Podocoryne selena*. Reproductive zooid with medusa buds in background. Scale bar equals 100 μm.

FIGURE 4. Reproductive zooids of *Podocoryne selena* with medusa buds. Note open mouth on upper polyp. Scale bar equals 100 μm.

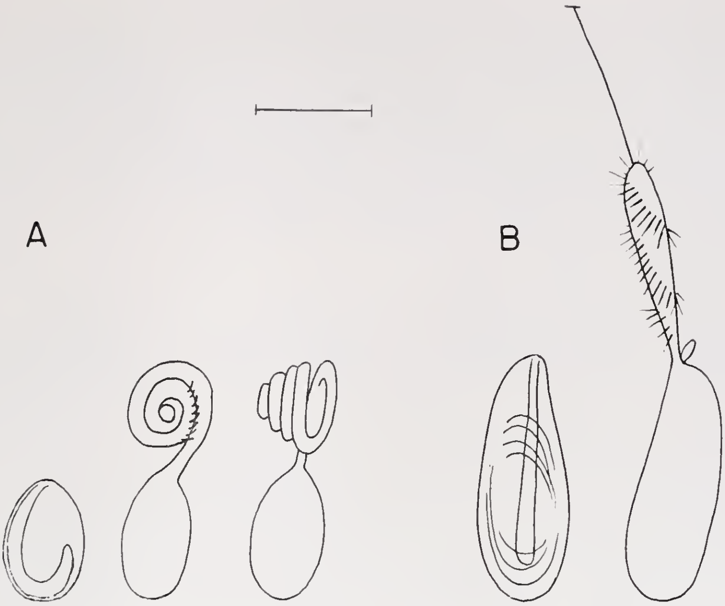


FIGURE 8. Nematocysts of *Podocoryne selena* and *Hydractinia echinata* from north Florida, drawn approximately to scale, as seen with phase contrast microscopy. The nematocysts of the two species are not morphologically differentiable. A. Desmonemes. B. Microbasic euryteles. Scale bar equals 5 μm.

Spines are usually present throughout the colony of *P. selena* on both live and hermit crab shells. Feeding zooids in colonies on hermit crab shells are generally located on the underside of the shell and reproductive zooids are located on the top and sides of the shell. Tentaculozooids were often seen at the edges of colonies in the laboratory that were either growing and expanding or regressing, the latter when insufficiently fed. On shells of the live gastropod *Cantharus*, feeding and reproductive polyps and spines were interspersed over the entire surface.

Type locality

Turkey Point, Franklin County, Florida (29° 56′ north latitude, 84° 30′ west longitude), in one meter of water.

Type material

The type specimen is an 11-day old female medusa with eggs. It has 13 tentacles and the preserved bell dimensions are 1.0 mm in both height and diameter.

FIGURE 5. Tip of reproductive zooid of *Hydractinia echinata* (similar to tip of spiral zooid of *H. echinata*). Scale bar equals 20 μm.

FIGURE 6. Spiral zooid of *Hydractinia echinata*. Feeding zooid can be seen in foreground. Scale bar equals 100 μm.

FIGURE 7. Reproductive zooids of *Hydractinia echinata* with fixed gonophores. Feeding zooid with long tentacles can be seen in center foreground. Scale bar equals 100 μm.

This holotype, preserved February 22, 1975, has been deposited at the National Museum of Natural History (Smithsonian Institution). Six lots of paratypes have also been deposited at the NMNH: (1) twenty-five newly released female medusae, (2) ten newly released male medusae, (3) ten 3-day old female medusae, (4) ten 3-day old male medusae, (5) fifteen 11-day old female medusae, (6) one polyp colony with medusa buds on a *Polinices duplicatus* shell containing the hermit crab *Pagurus pollicaris*. Additional paratype specimens have been deposited in the British Museum (Natural History), London.

Distribution and seasonal occurrence

Podocoryne selena polyps have been collected in Franklin County and Gulf County, Florida. It is probable that *P. selena* is found over a larger range, perhaps along most of the U. S. Gulf of Mexico coast. Whether *P. selena* is also found on the Atlantic coast of the U. S. has not yet been determined. Substrates for the polyps include the live horseshoe crab *Limulus polyphemus* (Shier, 1965), live *Cantharus cancellarius* shells, some dactyls and chelae of one specimen of the hermit crab *Pagurus pollicaris,* and the following hermit crab shells: *Busycon contrarium, Fasciolaria lilium hunteria, Murex pomum,* and *Pleuroploca gigantea,* containing the crab *Pagurus pollicaris*; and *Murex florifer dilectus, Polinices duplicatus,* and *Cantharus cancellarius* shells, containing the crabs *Pagurus pollicaris* or *Pagurus longicarpus.*

Podocoryne selena polyps have been collected every month except August and September in shallow subtidal (0–3 meters) waters in north Florida. Since robust, fully reproductive colonies have been collected at all other times, it is suspected that *Podocoryne selena* produces medusae year-round. Data that will be presented in a forthcoming paper discussing the interactions between these hydroids and hermit crabs in north Florida, indicates that conditions for planular settlement may become more favorable as spring progresses, since the number of young colonies increases considerably at that time.

Discussion

Edwards (1972) has reviewed the species of *Podocoryne* found in Britain which includes *P. carnea.* He notes that *P. carnea* has two forms in Europe, separated by a geographic cline of variation. The northern form seems to be similar to *P. carnea* in New England, although good descriptions of the developmental stages of medusae in New England are not available. *Podocoryne selena* is compared with northern and southern European forms of *P. carnea* as discussed by Edwards in Table II. Edwards speculates that the cline of variation observed in Europe for *P. carnea* may result from the longer life, larger size, greater numbers of tentacles, and slower sexual development in cooler waters. *Podocoryne selena* in the relatively warm waters of the Gulf of Mexico matures more rapidly, but also has more tentacles than *P. carnea. Podocoryne selena* also has far fewer exumbrellar nematocysts and fewer and larger eggs than *P. carnea,* and was never seen with a peduncle in any stage of development. *Podocoryne selena* medusae lived a maximum of 22 days in the laboratory, but their condition seemed to be deteriorating after the first two weeks. No data on *P. selena* in the plankton have been recorded.

TABLE II

Comparison of the medusae of northern and southern European Podocoryne carnea *and* Podocoryne selena. *Data for* P. carnea *are from Edwards (1972), Haeckel (1880), and Neppi (1917). Good descriptions of the developmental stages of* P. carnea *medusae in New England are not available.*

	P. carnea form *carnea* Northwest Europe and Britain	*P. carnea* form *exigua* Mediterranean (Naples)	*Podocoryne selena* north Florida
Newly released:			
Size	0.7–0.8 mm high	0.54 mm high	0.6–0.8 mm high
Number of tentacles	5–8 (mostly 6–7)	4 (rarely 5–7)	8 (rarely 5–7)
Condition of gonads	Usually present, rudimentary	Advanced	Advanced, almost ripe
Exumbrellar nematocysts	Many	?	Few
Adult medusa:			
Size	Usually 1.6–2.0 mm high maximum 2.4 mm high	0.8–1.2 mm high	Usually 1.0–1.5 mm high maximum 1.8 mm high
Number of tentacles	8 (some 7, 9)	4 (rarely 5)	8–14
Maturation of gonads	In 3 weeks	"Rapidly"	In 2 days

The medusae of *P. selena* are relatively inactive. Much of the time in the laboratory, they were seen on the bottoms of the culture dishes with tentacles partially extended, as in Figure 1A. Newly released medusae were more vigorous than older animals, swimming more of the time and often holding their tentacles contracted and curling up around the bell as in Figure 1D. The addition of *Artemia* nauplii to culture water containing starved medusae, caused the oral lips of the medusae to begin moving, each separately, in a groping fashion. When feeding, the medusae caught *Artemia* nauplii on their tentacles which were then contracted. The food particle was then stuffed through the small velar opening and was eventually contacted by the "groping" oral lips. The lips, consisting of batteries of large pendant microbasic euryteles (Fig. 2), maneuvered the food which was apparently being moved upwards into the stomach by muscular action, repeatedly grasping a little further down the length of the nauplius which was moved quite rapidly into the stomach. The medusae were capable of ingesting several nauplii in rapid succession. After catching 5 to 10 nauplii, the tentacles seemed to lose their ability to retain any further nauplii that they contacted, which suggests that few nematocysts were firing, similar perhaps to the results of Sandberg, Kanciruk, and Mariscal (1971) and Smith, Oshida, and Bode (1974). Nauplii that were contacted at this time showed little evidence of the immediate paralysis that occurred with the first-captured nauplii.

Specimens of both *Podocoryne selena* and *Hydractinia echinata* (Figs. 5–7) are found on hermit crab shells on the north Florida Gulf coast. In no instances have both species of hydroid been found on the same shell, although a few shells with *Hydractinia* appeared to have two colonies, separated by a distinct boundary, living

TABLE III

Differences between the hydroids Podocoryne selena *and* Hydractinia echinata.

Character	*Podocoryne selena*	*Hydractinia echinata*
Gonophores	Releases free medusae	Fixed, releasing eggs or sperm directly into water
Spines	Smooth	Usually, but not always, jagged
Spiralzooids	Have no tentacles, nematocysts especially dense near the tip	With stubby tentacles in a distal whorl, which serve as nematocyst batteries
Gonozooids	With tentacles similar to, but in lesser number than, nutritive zooids	With reduced stubby tentacles similar to those of spiralzooids
	Gonophores borne in whorled cluster below tentacles	Gonophores borne in whorled cluster below tentacles

on the same shell. In general, either species of hydroid might be found on any hermit crab shell in north Florida occupied by crabs in the genus *Pagurus* (the crab *Clibanarius vittatus* does not occupy hydroid-covered shells). There seem to be no clear cut preferences by either species of hydroid for any species of shells. In contrast to this situation in Florida, Crowell (1945) reports that in the vicinity of Woods Hole, in New England, where either *P. carnea* or *H. echinata* might be found on several species of hermit crab shells with apparently little demonstration of specificity, only *P. carnea* is found on *Nassa trivittata* shells, and *Littorina littorea* shells almost always support colonies of *H. echinata.*

The species *Podocoryne* and *Hydractinia* are closely related, both belonging to the family Hydractiniidae, but are easily distinguished when the colonies bear gonophores. *Podocoryne* releases free medusae whereas *Hydractinia* bears fixed gonophores which release gametes directly into the sea. Table III lists some easily recognizable difference between *P. selena* and *H. echinata.* The cnidoms are quite similar and are given in Table I and illustrated in Figure 8. Both species have desmonemes and microbasic euryteles.

It is interesting to note that the range of *H. echinata* closely corresponds to that of *P. carnea.* Both are found in north European waters, the Mediterranean Sea, and the north Atlantic coast of North America. *Hydractinia echinata* is also described from the Sea of Japan and more northern Pacific and Arctic waters (Naumov, 1969). Since *P. selena* seems to replace *P. carnea* in warmer water, it is possible that the *Hydractinia echinata* in the Gulf of Mexico is not the same species as the temperate and boreal form of *H. echinata,* but there is no readily observable characteristic like the medusae of *Podocoryne* that clearly suggests this hypothesis.

Spiral zooids are found in some species in the family Hydractiniidae, including *P. carnea, P. selena,* and *H. echinata,* on hermit crab shells. The function of spiral zooids in the polyp colonies is unclear. They are commonly believed to serve a defensive function for the hydroid colony, as is suggested by their morphology and the distribution of nematocysts on their surfaces (see Figs. 3 and 6). Spiral zooids respond to movement of the hermit crab shell, but not specifically to tactile stimulation to the spiral zooid. By jostling a hermit crab shell briefly, this author was able to make the spiral zooids lash out simultaneously and repeatedly, but similar behavior was not observed during extensive hermit crab shell changing experiments involving three species of hermit crabs (*Pagurus pollicaris, Pagurus longicarpus,* and *Clibanarius vittatus*) and hydroid-covered shells. Schijfsma (1935) also attempted to discover the purpose of spiral zooids in *H. echinata* in Europe. After extensive experimentation, she could find no explanation of their purpose and no evidence that they were a special adaptation of the hydroid that served to benefit the host hermit crab, as has been suggested. Wright (1973) also does not report any special defensive behavior by *H. echinata* or *P.* "*carnea*" spiral zooids, nor does M. Braverman (Taos, New Mexico, personal communication). Braverman (1974) found that spiral zooids form in response to the movements of the hermit crabs in and out of the shells. Burnett, Sindelar, and Diehl (1967) hypothesized that some diffusable material produced by the hermit crab was a contributing factor to the growth of spiral zooids on the shell. The purpose of spiral zooids thus remains an intriguing problem.

The arrangement of polyps in *Podocoryne selena* and *Hydractinia echinata* colonies in Florida is such that the nutritive polyps are situated on the underside of the shell and are dragged over the sand as the hermit crab moves. In a similar situation in Denmark, Christensen (1967) found, by analyzing the stomach contents of *Hydractinia echinata* polyps growing on the shells of the hermit crab *Pagurus bernhardus,* that the hydroid was deriving 90% of its food from the benthos, eating primarily nematodes, ophiuroid arm joints, and harpacticoid copepods. No food analysis has been done on *P. selena* or *H. echinata* in Florida, but they presumably feed in the same manner.

My appreciation is extended to Richard Mariscal, Cadet Hand, and James Carlton for reading the manuscript and offering useful discussion and suggestions. Thanks are due the Electron Microscope Laboratory of the Florida State University for the use of facilities. A portion of this work was supported by NSF grant #GB-40547 to R. N. Mariscal.

SUMMARY

1. A new species of hydroid, *Podocoryne selena,* is described from the north Florida Gulf of Mexico. This hydroid has mistakenly been called *P. carnea* in the past; polyps are very similar in the two species. It cannot be determined whether *P. carnea* actually occurs in subtropical waters until mature medusae are examined in other regions.

2. Observations on the behavior of the polyps and medusae of *P. selena* are given.

3. The species *Podocoryne selena* and *Hydractinia echinata* are both found living on hermit crab shells in the Gulf of Mexico. The two species are compared and morphological differences are illustrated with scanning electron micrographs.

LITERATURE CITED

BRAVERMAN, M., 1974. The cellular basis of morphogenesis and morphostasis in hydroids. *Oceanogr. Mar. Biol. Annu. Rev.*, **12**: 129–221.

BURNETT, A. L., W. SINDELAR, AND N. DIEHL, 1967. An examination of polymorphism in the hydroid *Hydractinia echinata*. *J. Mar. Biol. Ass. U. K.*, **47**: 645–658.

CARY, L. R., AND M. H. SPAULDING, 1909. Further contributions to the fauna of the Louisiana coast. *Bull. Gulf Biol. Sta.*, **12**: 1–21.

CHRISTENSEN, H. E., 1967. Ecology of *Hydractinia echinata* (Fleming) (Hydroidea, Athecata). I. Feeding biology. *Ophelia*, **4**: 245–275.

CROWELL, S., 1945. A comparison of shells utilized by *Hydractinia* and *Podocoryne*. *Ecology*, **26**: 207.

DEEVEY, E. S. JR., 1950. Hydroids from Louisiana and Texas, with remarks on the Pleistocene biogeography of the western Gulf of Mexico. *Ecology*, **31**: 334–367.

DEEVEY, E. S. JR., 1954. Hydroids of the Gulf of Mexico. *U. S. Fish Wildlife Serv. Fish. Bull.*, **89**: 267–272.

DEFENBAUGH, R. E., AND S. H. HOPKINS, 1973. *The occurrence and distribution of the hydroids of the Galveston Bay, Texas, area.* Center for Marine Resources, Texas A and M University, 202 pp.

EDWARDS, C., 1972. The hydroids and medusae *Podocoryne areolata, P. borealis* and *P. carnea*. *J. Mar. Biol. Ass. U. K.*, **52**: 97–144.

HAECKEL, E., 1880. *Das System der Medusen.* Erster Teil, Zweite Hälfte, Jena, Gustav Fischer, 634 pp.

JOYCE, E. A., JR., 1961. The Hydroida of the Seahorse Key area. *M. S. Thesis, University of Florida*, Gainesville, Florida, 116 pp.

MARISCAL, R. N. 1974a. Nematocysts. Pages 129–178 in L. Muscatine, Ed., *Coelenterate biology: reviews and new perspectives.* Academic Press, New York.

MARISCAL, R. N., 1974b. Scanning electron microscopy of the sensory epithelia and nematocysts of corals and a corallimopharian sea anemone. *Proc. Second Int. Coral Reef Symposium*, **1**: 519–532.

McLEAN, R. B., 1975. A description of a marine benthic faunal habitat web: a behavioral study. Ph.D. Dissertation, Florida State University, Tallahassee, Florida, 176 pp. (*Diss. Abstr.*, 36(B) : 2567, order number 75–26, 795.)

MENZEL, R. W., 1971. *Checklist of the marine fauna and flora of the Apalachee Bay and the St. George's Sound area.* Dept. of Oceanography, Florida State University, 126 pp.

NEPPI, V., 1917. Osservazioni sui polipi idroidi del golfo di Napoli. *Pubbl. Staz. Zool. Napoli*, **2**: 29–65.

NAUMOV, D. V., 1969. *Hydroids and hydromedusae of the USSR.* Israel Program for Scientific Translations, Jerusalem, 660 pp.

PARDUCZ, B., 1967. Ciliary movement and coordination in ciliates. Pages 91–128 in G. H. Barnes and J. F. Danielli, Eds., *International review of cytology, Vol. 21.* Academic Press, New York.

SANDBERG, D. M., P. KANCIRUK, AND R. N. MARISCAL, 1971. Inhibition of nematocyst discharge correlated with feeding in a sea anemone, *Calliactis tricolor* (LeSeur). *Nature*, **232**: 263–264.

SCHIJFSMA, K., 1935. Observations on *Hydractinia echinata* (Flem.) and *Eupagurus bernhardus* (L.). *Arch. Neerlandaises Zool.*, **1**: 261–314.

SHIER, C. F., 1965. A taxonomic and ecological study of shallow water hydroids of the northestern Gulf of Mexico. *M. S. Thesis, Florida State University*, Tallahassee, Florida, 128 pp.

SMITH, S., J. OSHIDA, AND H. BODE, 1974. Inhibition of nematocyst discharge in *Hydra* fed to repletion. *Biol. Bull.*, **147**: 186–202.

WELLS, H. W., 1969. Hydroid and sponge commensals of *Cantharus cancellarius* with a "false shell." *Nautilus*, **82**: 93–102.

WRIGHT, H. O., 1973. Effect of commensal hydroids on hermit crab competition in the littoral zone of Texas. *Nature*, **241**: 139–140.

Reference: *Biol. Bull.,* **151**: 225–235. (August, 1976)

THE PRODUCTION OF APOSYMBIOTIC HYDRA BY THE PHOTODESTRUCTION OF GREEN HYDRA ZOOCHLORELLAE

R. L. PARDY

Department of Developmental and Cell Biology, University of California, Irvine, California 92664

Research progress and continued interest in the hydra-algae symbiosis is, to a great extent, owed to the existence of aposymbiotic animals. Aposymbiotic hydra are hydra that normally harbor green, *Chlorella*-like algae in their digestive cells but through circumstances described below, have become algal-free. Aposymbiotic hydra survive and reproduce as well as green animals, providing they are fed regularly (Muscatine and Lenhoff, 1965), and serve as important control and experimental organisms in many experiments examining the physiology and morphology of the symbiosis. For instance, experiments regarding the nutritional basis of the symbiosis (Muscatine and Lenhoff, 1965), investigations demonstrating the recognition of potential symbionts hydra (Pardy and Muscatine, 1970, 1973), studies detailing the ultrastructural aspects of the animal-algal association (Pardy, unpublished), and energetic evaluation of the symbiosis (Stiven, 1965), have all been possible due to the availability of aposymbiotic hydra.

While the existence of free-living aposymbiotic hydra in nature is unreported, algal-free animals occasionally arise in laboratory cultures as a result of the chance occurrence of an algal-free zygote (Lenhoff, 1965). Such aposymbiotic zygotes mature and by asexual budding, produce clones of white animals that do not normally become symbiotized in laboratory cultures. These aposymbionts, however, can be reinfected artificially with symbiotic algae (Pardy and Muscatine, 1973) and thus retain the capacity for symbiosis.

The occurrence of algal-free zygotes, however, is at best an uncertain and capricious process. Moreover, the production of hydra by sexual processes gives rise to genetic recombinants which may not have the same physiological characteristics as the asexually reproducing parent clone. Moore and Campbell (1973) have shown that inbred hydra give rise to zygotes that exhibit high mortality and that some zygotes exhibit morphological and developmental aberrations. The latter condition was first described by Lenhoff (1965), who discovered a mutant strain of nonbudding hydra that was produced by sexual processes.

Objections to the use of aposymbiotic hydra derived from algal-free zygotes described above are partly overcome by the production of aposymbiotic animals in the laboratory by chemically treating adult, green hydra. By means of a technique first described by Whitney (1907), green hydra are maintained in 0.5% glycerine. After several days of exposure to glycerine, some animals become pale green to white. From some of the white animals, clones of aposymbiotic hydra can be reared by means of asexual budding. Using this method, investigators are able to prepare routinely algal-free animals that are genetically identical to their symbiotized progenitors. Glycerination has been used to produce aposymbiotic hydra from the following strains of *Chlorohydra viridissima*: Carolina (Muscatine and Lenhoff, 1965), Burnett (Park, Greenblatt, Mattern and Merril, 1967) and from *C. viridissima* of unknown origin (Stiven, 1965). In addition, Epp and Lytle (1969) have prepared algae-free specimens of *Chlorohydra hadleyi* using glycerine.

Not all green hydra are amenable to glycerination. Unsuccessful attempts to produce aposymbiotic hydra have been reported for *C. viridissima* Burnett strain (Epp and Lytle, 1969) and Florida strain *C. viridissima* (Muscatine, 1974). Moreover, Muscatine (1974) reports resistance to glycerination by a strain of hydra designated European; and in unpublished experiments, I have been unable to produce aposymbients from *Hydra viridis*, Florida strain or an English strain of green hydra.

In this paper, a new method of aposymbiont formation is described, involving the apparent photodestruction of symbionts, that is successful in obtaining clones of algal-free hydra from strains refractory to glycerination.

Materials and Methods

Experimental animals

Laboratory populations of the green hydra species, *Hydra viridis* (Florida strain) and a larger strain of green hydra, designated the English strain, were reared and maintained in M solution according to the methods of Lenhoff and Brown (1970). The English animals, a gift of Dr. L. Muscatine, are easily distinguished from the Florida hydra by their larger size and characteristic nematocyst dimensions. Mature, budding English hydra average a relaxed length of about 3–3.5 mm *vs.* a length of 2–2.5 for the Florida animals under similar conditions. The tentacle stenoteles of the English strain average 8.7 × 10.4 microns *vs.* 7 × 9 microns for the stenoteles of the Florida strain. Length and nematocyst dimensions are from personal, unpublished observations.

The animals used in experiments were harvested from logarithmically growing populations fed daily on freshly hatched *Artemia* nauplii and maintained at 18 ± 1° C in a photoperiod incubator under a 12 hour light/dark regime. Only mature animals possessing one to three buds were used.

Irradiation of animals with intense light

Animals to be exposed to high light intensity were placed in 70 ml plastic tissue culture bottles filled with M solution. Each vessel was completely submerged in a transparent bath maintained at 15 ± 1° C and continuously illuminated by 150 watt G. E. reflector flood lamps. The amount of radiation impinging on the culture bottles was controlled by varying the distance of the lamp above the experimental cultures. The amount of light energy reaching the surface of the culture bottles was measured using a Yellow Springs Model 65A radiometer. A spectral analysis of the lamps used as light sources was performed using an ISCO spectroradiometer. During some irradiation experiments, green hydra were simultaneously exposed to 10^{-6} M DCMU [3-(3,4-dichlorophenyl)-1,1-dimethylurea, from K and K Laboratories, Inc., Plainview, New York]. DCMU is a specific photosynthetic inhibitor (Losada and Arnon, 1963) which interferes with the oxygen evolving photochemical system of chloroplasts.

In some experiments, animals were exposed to light that had passed through Baush and Lomb medium band-pass filters (numbers 90-4-460, 90-4-450, 90-4-620).

Examination of animals for the presence of symbiotic algae

Hydra were analyzed for the presence of algal symbionts by direct examination of animals under a 30× dissecting microscope. Animals exhibiting no trace of green color in any part of their bodies (column, tentacles, peduncle, buds) when examined against a white background were scored as "bleached." Alternatively, direct examination of digestive cells for the presence of algae was done by macerating whole animals (David, 1973). Maceration of intact animals were accomplished by placing hydra in 0.2–0.5 ml of a solution consisting of glycerine, glacial acetic acid and water (1:1:13, V/V). After 15–30 minutes the preparation was gently shaken, a drop of 15% formalin added and wet slides prepared of the resulting cells. Slides were examined using phase optics.

Spectrophotometric analysis

Green and aposymbiotic hydra tissues were assayed for the presence of pigments by extraction of 10–20 whole animals in 2 ml of absolute ethanol. The animals were extracted at 4° C for 24 hours, after which the methanolic extracts were scanned in the visible range (400–700 nm) using either a Beckman Acta II or Cary 14 dual beam scanning spectrophotometer.

Experimental cross infection of algal symbionts

In some experiments, English aposymbiotic hydra infected with Florida symbionts and Florida aposymbiotic animals infected with symbionts originating from English green animals were used. The infection of aposymbiotic animals is a simple procedure involving homogenation of the animal donors in M solution followed by the separation and purification of the algae via low speed centrifugation of host homogenate and repeated washing of the algae cells.

Dense slurries of symbionts are injected directly into the recipient hosts enteron by means of a glass micropipette. Following injection, the animals are maintained under normal culture (ambient light, daily feeding) conditions and are considered to be repopulated when they are green through the body column and tentacles—a process that takes about 10–15 days. Specific details of algal injection and aposymbiont repopulation are given in Pardy and Muscatine (1973).

RESULTS

Green Florida and English strain hydra were initially exposed to light of 620 watts/m^2. After five days of continuous exposure, the animals were analyzed for the presence of green color as described earlier. Table I shows that nearly half (49.5%) of the Florida animals appeared bleached whereas none of the English animals became white.

I have used DCMU before in unpublished work in an attempt to rid English and Florida hydra of this algal symbionts but without success. In the present work another attempt was made, only this time coupling the exposure of the animals to the inhibitor while simultaneously irradiating the animals at 620 watts/m². Under these conditions (with DCMU) almost all (99.3%) of the Florida hydra and a third (32.8%) of the English hydra appeared bleached (Table I).

As the English animals did not visibly bleach at 620 watts/m² in the absence of DCMU, in another experiment these animals and Florida strain hydra were subjected continuously to 1900 watts/m². Under these conditions all Florida animals were bleached at 72 hours at which time 62.5% of the English hydra were also bleached (Table I). Attempts to increase the irradiance further or to prolong exposure beyond 72 hours at 1900 watts/m², resulted in the disintegration of many of the animals in both strains.

To determine if the observed decrease in green color (=bleached) was due to a loss of symbionts and to evaluate the condition of the algae, samples of digestive cells from hydra were examined prior to irradiation and from animals judged bleached after five and seven days of continuous exposure to 620 watts/m² with or without DCMU. Table II shows the results of this experiment, and it is evident that the observed bleaching of the Florida strain hydra (with or without DCMU) and the English strain with DCMU results from a precipitous drop in the number of algal symbionts over the first five days. Algae continued to be lost from digestive cells in the 5–7 day interval with an increase in the number of cells lacking symbionts. Loss of algae from the Florida and English strains of hydra was most pronounced in those animals treated with DCMU (Table II) with all cells being void of symbionts in Florida strain (*vs.* 72% without algae in the absence of DCMU) and 95% of the digestive cells with no symbionts in the English strain (*vs.* 0% without algae in the absence of DCMU).

Within the digestive cells, the algal symbionts exposed to light at 620 watts/m² with or without DCMU exhibited a characteristic appearance. Compared with untreated controls, symbionts remaining in bleached animals appeared to be brownish in color and to be internally disrupted. Moreover the algae were located at the distal ends of the host's digestive cells sequestered in a large apical vacuole.

The results described above show that light and light DCMU are effective in causing the visual bleaching of green hydra. Moreover, microscopical analysis has shown that the observed loss of green color is due to elimination of the algal symbionts from the host's digestive cells. To see how complete and persistent the bleaching treatments were, 36 bleached animals of both strains were maintained under ambient light conditions and fed every three days. After three weeks the cultures were assayed for the presence of bleached or algal-containing hydra. The results of this experiment are shown in Table III. Almost all of the bleached Florida hydra (98.5%) remained algal free (100% in DCMU) and gave rise to aposymbiotic offspring by means of asexual budding. The bleached animals produced with or without DCMU showed no observable ill effects. The bleached English animals showed a lower proportion of permanently bleached animals (72.0%) indicating that the bleaching process had probably not been complete and that viable symbionts remained to reestablish the algal population. From both populations (Florida and English) clones of aposymbiotic hydra have been reared, the algal-free progney of which exceeds several thousands.

While both strains of hydra became bleached at 1900 watts/m², only the Florida strain produced aposymbiotic animals at 620 watts/m². To determine if the in-

TABLE I

The effect of various treatments on the "bleaching" of green hydra.

Hydra strain and experimental conditions	Number of animals bleached	Number of animals not bleached	Per cent bleached
Florida			
5 days, 620 watts/m^2	76	77	49.5
5 days, 620 watts/m^2 + DCMU	128	1	99.3
72 hours, 1900 watts/m^2	24	0	100.0
Florida			
3 days, 703 watts/m^2	0	24	0
460 nm band-pass filter			
3 days, 741 watts/m^2			
540 nm band-pass filter	0	24	0
3 days, 741 watts/m^2			
620 nm band-pass filter	15	9	62.5
English			
5 days, 620 watts/m^2	0	148	0
5 days, 620 watts/m^2 + DCMU	45	92	32.8
72 hours, 1900 watts/m^2	15	9	62.5
Florida hosts containing English symbionts—5 days, 620 watts/m^2	42	58	42.0
English hosts containing Florida symbionts—5 days, 620 watts/m^2	0	203	0

ability to bleach English animals at the lower irradiance was a function of symbiont resistance or host factors, English aposymbiotic recipients were cross infected with algae from Florida donors and likewise were infected with algae from English donors. After repopulation of the recipient hosts was complete, the animals were exposed to continuous irradiance of 620 watts/m^2. Table I shows that Florida hydra containing algae from English donors become bleached and the effectiveness of the treatment (42.0%) approaches that exhibited by the Florida animals when symbiotized by their native algae (49.5%). As before (Table I) English hydra, even though infected with algae from the Florida strain, did not become bleached, nor were any bleached individuals observed in this culture after 15 days of continuous exposure to irradiance of 620 watts/m^2.

To further explore the role of light in the bleaching phenomenon, Florida strain green hydra were exposed to light passed through medium band-pass filters as described in Materials and Methods. In each of the three experiments, the light source was adjusted to deliver a comparable amount of energy, though in one experiment involving a filter with a half-band width of 460 nm, heat problems necessitated an irradiance (703 watts/m^2) of approximately 6% less than that used with the other filters (741 watts/m^2). This small difference was considered insignificant. The high radiant energies required to bleach English hydra made it impractical to perform this series of experiments on these animals. Table II shows the results of these experiments and indicates that light composed mainly of the longer (red) wavelengths is most effective in causing bleaching of Florida hydra.

TABLE II

*Number of algal symbionts per digestive cell and digestive cells with no algae in Florida and European strains of hydra following five and seven days continuous exposure to light of 620 watts/m² with or without DCMU.**

Hydra strain	Day 0	Day 5		Day 7	
	Average number algae per digestive cell	Average number algae per digestive cell	Per cent of cells with no algae	Average number algae per digestive cell	Per cent of cells with no algae
Florida	15.98 ± 6.17	1.15 ± 2.30	67	1.09 ± 1.93	72
English	20.34 ± 6.38	19.42 ± 7.90	0	17.50 ± 7.15	0
Florida + DCMU	15.98 ± 6.17	0.07 ± 0.90	96	0	100
English + DCMU	20.34 ± 6.38	1.32 ± 2.52	69	0.21 ± 0.95	95

* Digestive cells were prepared by macerating 10 hydra together and counting the algae in 100 randomly selected digestive cells. Data expressed as mean ± standard deviation.

The spectral characteristics of the filters and the illumination source used in all experiments are shown in Figure 1A and B. Figure 1B shows that the unfiltered source yielded light predominately in the region passed by the red-band filter (550–650).

Spectrophotometric analysis of methanolic extracts of Florida and English green hydra revealed two distinct regions of light absorption: 400–500 nm and 580–680 nm. The absorption spectra for the two strains were identical and the curve for the Florida strain is shown in Figure 1C. Aposymbionts exhibited one peak at approximately 475 nm.

There existed the possibility that the epidermal cells of the green hydra act as light filters and that their efficiency as light screens would be a function of their thickness. Thus (as described earlier) 25–30 hydra of each strain were macerated and the cell thickness (length) of 50 randomly selected epidermal cells was measured using an eyepiece micrometer and phase optics. From these measurements, it could be determined that the English hydra had epidermal cells averaging 37 ± s.d. 6 μ thick, compared to the Florida animals which had cells averaging 25 ± s.d. 7 μ thick.

DISCUSSION

The results of the experiments described in this paper show that the symbiotic algae in two strains of green hydra (Florida and English) may be eliminated by exposing the hosts to intense light. I have called this phenomenon "bleaching." Tables I and II show that with time the animals exposed to strong light appear pale and that their bleached condition is due to the elimination of symbiotic algae from the host's digestive cells. Many of the bleached animals remain algal-free (Table III) and give rise to clones of aposymbiotic hydra. These aposymbionts are still receptive to algal symbionts as they can be reinfected with algae harvested from green hydra. Of interest is the fact that algae from different strains of hydra can be interchanged between different hosts. In the present work, English symbionts were successfully transferred to Florida hosts and *vice versa*. Apparently, both hydra strains "recognize" potential algal symbionts. Recognition, uptake, and rejection of algae by symbiotic hydra have been previously investigated (Pardy and

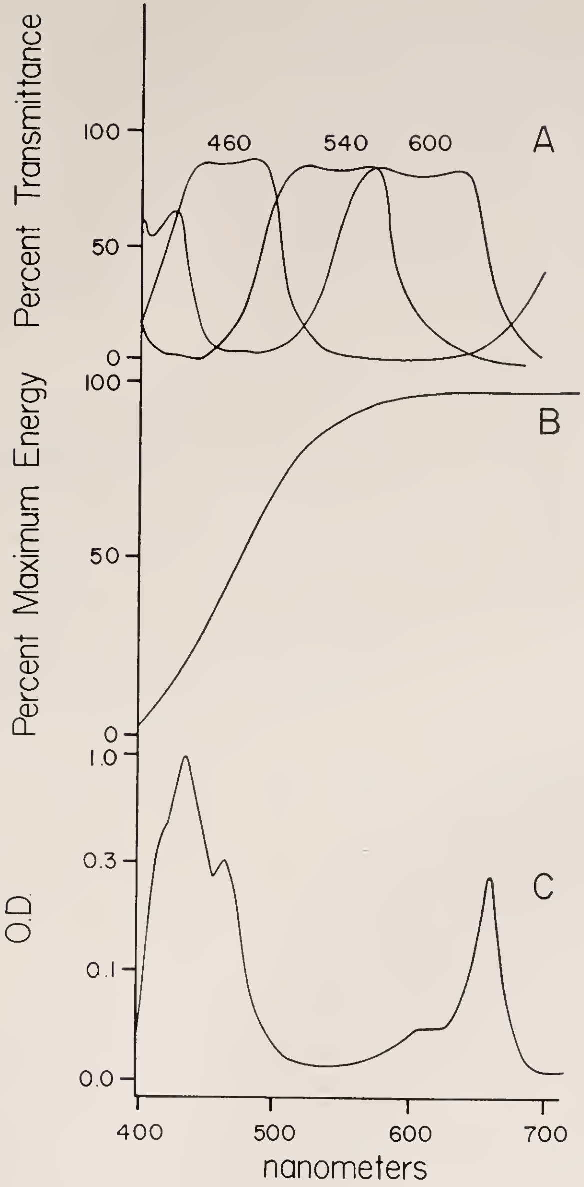


FIGURE 1. Curves of spectral absorbance of three medium band-pass filters used in experiments (A), spectral energy of light used in bleaching experiments (B), and optical density of a methanolic extract of green hydra (Florida strain) (C), plotted as a function of wavelength. The curves in (A) are reprinted from technical data sheets supplied by Baush and Lomb with permission.

TABLE III

Success of various bleaching treatments as measured by the number of bleached and green animals in a population of hydra three weeks post treatment.

Hydra strain and experimental treatment	Number of bleached hydra	Number of green hydra	Per cent remaining bleached
Florida			
5 days, 620 watts/m²	67	1	98.5
5 days, 620 watts/m² + DCMU	66	0	100.0
English			
5 days, 620 watts/m² + DCMU	140	45	72.0

Muscatine, 1973). In this work (Pardy and Muscatine, 1973), it was shown that hydra can recognize potential symbionts and reject non-symbiotic algae. It is possible that the symbionts resident in the Florida and English strains of green hydra are identical; however, in a study recently completed (Pardy, 1976), it was found that the Florida and English symbionts may be distinguished on the basis of their ultrastructure.

From Table I it is evident that the English strain of hydra (or their algae) is less sensitive to the effects of intense light than the Florida animals in 620 watts/m^2 but is bleached when the irradiation is increased (Table I). Evidence derived from the cross transfer of algae between the two host strains suggests that the apparent resistance of the English algae to the effects of irradiance at 620 watts/m^2 is a function of the host in which it is resident (Table III). When algae are in Florida hosts, they are subject to the effects of light at 620 watts/m^2 while Florida algae in English hosts appear to have gained immunity. The bleaching of European hosts that takes place at 1900 watts/m^2 suggests that the protective mechanism existing in these animals may be overridden. While the nature of the protective mechanism is not known exactly, it may be related to the larger cell size evidenced by the European animals. To reach the endosymbiotic algae, light in the external environment must traverse the epidermal epithelium which may act as a light screen. The thickness of these cells, which in the European hosts is 1.3 times that of the Florida animals, may be the basis for the failure of the English animals to bleach at the lower irradiance.

The role of high intensity light in causing elimination of algal symbionts is not thoroughly understood, though there are at least two possible explanations. Unfavorable conditions such as elevated temperature and starvation have been shown to cause corals and sea anemones to rid themselves of symbionts (Muscatine, 1974). Likewise, intense light might act directly on the animal tissue inducing some unfavorable physiological condition causing the host to expel its symbionts. That high intensity light may adversely affect the animals was indicated by the disintegration of hydranths (Florida and English) when exposed to 1900 watts/m^2 for periods exceeding 72 hours. At this high intensity, however, the death and subsequent release of metabolites by a greater number of moribund algae cannot be completely ruled out as a factor in causing the demise of the hydra.

Alternatively intense light could act directly on the algal symbionts to cause the destruction of photosynthetic pigments or other components of the photosynthetic apparatus. When chlorophyll pigment in algae becomes oversaturated with light quanta, it is degraded and the enzymatic systems associated with carbon dioxide fixation are inactivated (Steemann-Nielsen, 1962). The photodestruction of chlorophyll, however, takes place predominantly in the blue region of the spectrum (Soeder and Stengel, 1974) which is ineffective (Table I) in causing the bleaching of green hydra. The experiments shown in Table I and the measurements depicted in Figure 1 clearly implicate light in the red portion of the spectrum as being the active principle in the bleaching phenomenon. Absorption measurements on green hydra extracts reveal peaks (Fig. 1C) that are characteristic of green algae chlorophylls which absorb maximally in two regions—400–500 nm and 600–700 nm (Bogard, 1962).

Thus, while the chlorophyll pigments may not be undergoing photodestruction, it is possible that some red absorbing component associated with photosynthesis is being degraded. Further support for this argument follows from the observation that DCMU hastens the bleaching of Florida hydra and is essential for the elimination of algae from English hydra at 620 watts/m^2. DCMU, a specific photosynthetic inhibitor, has been shown to cause the bleaching of chlorophyll pigments as well as inhibit oxygen evolution and $^{14}CO_2$ incorporation (Zweig, Hitt and Cho, 1969). Recently Pardy and Dieckman (1975) have shown that DCMU inhibits photosynthesis of symbiotic algae *in situ*. It must be added that in unpublished experiments, I have been unable to cause bleaching with DCMU at ambient levels of light usually employed during culture of green hydra.

Finally, the disrupted appearance of the symbionts, when viewed under the microscope tends to suggest that intense light acts on the algae directly. These degraded algae are in striking contrast to the otherwise normal appearing host digestive cells within which they are contained.

Following photodestruction, the algal symbionts are removed from the host's digestive cells probably by emiocytosis. Once in the coelenteron, the algae are swept out *via* the water currents associated with respiration and elimination. In previous work (Pardy and Muscatine, 1973) it was shown that heat-inactivated symbionts were expelled from digestive cells following their localization in a large apical vacuole. Viable symbionts normally reside in individual vacuoles (Oshman, 1967) located at the base of the digestive cell. The expulsion of algae following exposure to high intensity light appears to be a process similar to the removal of heat-inactivated algae. How the host cell recognizes dead or moribund cells and moves them from the base of the cell to the apex for elimination is unknown. In a review, Muscatine (1974) cites his unpublished observations on algae in hydra treated with glycerine. According to Muscatine (1974), glycerine brings about the degradation of symbiotic algae followed by their elimination from the host. Hence the removal of pathologic symbionts appears to be a generalized response in green hydra, though the mechanism is unknown.

It is now possible to prepare aposymbiotic green hydra from strains refractory to glycerination. Moreover I have been able to produce aposymbionts of the English strain—a strain from which the existence of aposymbionts has yet to be reported. The importance of these algal-free English animals cannot be overstressed as these hydra (green and aposymbiotic) differ morphologically from the other strains as described earlier (see also Muscatine, 1974) and, from unpublished observations, exhibit a variety of physiological characteristics (growth rate, metabolic rate, phototaxis) different from other green hydra.

With the advent of aposymbiotic forms of this strain together with the ability to cross-infect it with algae from other strains, new research avenues into the hydra-algae symbiosis are possible. For instance, in a recent work (Pardy, 1976), the ultrastructure of the algal symbionts residing in the English hydra were found to differ from that of the Florida symbionts. By making reciprocal algal crosses into aposymbionts prepared by bleaching, it could be seen that the host strain may determine the morphology exhibited by the algal symbionts. Such studies were made possible by the ability to produce English aposymbionts.

To the list of methods which give rise to aposymbiotic hydra from green animals (algal-free zygotes, glycerination) can now be added the photodestruction of symbiotic algae. How widely applicable this technique is to other strains of hydra or to other symbiotic species is not known although its use is presently being investigated on symbiotic protozoa and sea anemones.

SUMMARY

1. Florida strain, but not the English strain of green hydra, are bleached by light at 620 watts/m^2.

2. English strain animals are bleached at 620 watts/m^2 in the presence of DCMU (a photosynthetic inhibitor) which also increases the bleaching effect in Florida hydra. English animals are also bleached by irradiation with 1900 watts/m^2.

3. Aposymbiotic clones that remain algal-free may be grown from bleached animals of both Florida and English strains.

4. Florida strain hydra containing English algae are bleached at 620 watts/m^2 but English hydra containing Florida algae are not.

5. The bleaching of Florida hydra takes place with light occurring in red region of the visible spectrum and probably involves the photodestruction of the photosynthetic system of the algal symbionts.

6. The bleaching of green hydra ultimately results from the removal of symbionts from the host's digestive cells.

LITERATURE CITED

Bogard, L., 1964. Chlorophylls. Pages 385–408 in R. A. Lewin, Ed., *Physiology and biochemistry of algae*. Academic Press, New York and London.

David, C. N., 1973. A quantitative method for maceration of hydra tissue. *Willhelm Roux Arch. Entw. Mech. Org.*, **171**: 259–268.

Epp, L. G., and C. R. Lytle, 1969. The influence of light on asexual reproduction in green and aposymbiotic hydra. *Biol. Bull.*, **137**: 79–94.

Lenoff, H. M., 1965. Cellular segregation and heterocyte dominance in hydra. *Science*, **148**: 1105–1107.

Lenoff, H. M., and R. Brown, 1970. Mass culture of hydra: an improved method and its application to other aquatic invertebrates. *Laboratory Animals*, **4**: 139–154.

Losada, M., and D. I. Arnon, 1963. Selective inhibitors of photosynthesis. Pages 559–593 in P. M. Hochester and J. H. Quastel, Eds., *Metabolic inhibitors, Vol. 2.* Academic Press, New York.

Moore, L. B., and R. D. Campbell, 1973. Non-budding strains of hydra: isolation from sexual crosses and developmental regulation of form. *J. Exp. Zool.*, **185**: 73–81.

MUSCATINE, L., 1974. Endosymbiosis of cnidarians and algae. Pages 359–395 in L. Muscatine and H. M. Lenhoff, Eds., *Coelenterate biology: reviews and new perspectives.* Academic Press, New York.

MUSCATINE, L., AND H. M. LENHOFF, 1965. Symbiosis of hydra and algae. II. Effects of limited food and starvation on growth of symbiotic and aposymbiotic hydra. *Biol. Bull.*, **128**: 415–424.

OSHMAN, J. L., 1967. Structure and reproduction of the algal symbionts of *Hydra viridis. J. Phycol.*, **3**: 221–228.

PARK, H. D., C. L. GREENBLATT, C. F. T. MATTERN, AND C. R. MERRIL, 1967. Some relationships between *Chlorohydra,* its symbionts and some other chlorophyllous forms. *J. Exp. Zool.*, **164**: 141–162.

PARDY, R. L., 1976. The morphology of green hydra endosymbionts as influenced by host strain and host environment. *J. Cell Sci.*, **20**: 655–669.

PARDY, R. L., AND C. L. DIECKMAN, 1975. Oxygen uptake by the symbiotic hydra, *Hydra viridis. Exp. Zool.*, **194**: 373–378.

PARDY, R. L., AND L. MUSCATINE, 1970. Recognition of symbiotic algae by *Hydra viridis. Amer. Zool.*, **10**: 513.

PARDY, R. L., AND L. MUSCATINE, 1973. Recognition of symbiotic algae by *Hydra viridis.* A quantitative study of the uptake of living algae by aposymbiotic *H. viridis. Biol. Bull.*, **145**: 565–579.

SOEDER, C., AND E. STENGEL, 1974. Physio-chemical factors affecting metabolism and growth rate. Pages 717–718 in W. D. P. Stewart, Ed., *Algal physiology and biochemistry.* University of California Press, Berkeley and Los Angeles.

STEEMANN-NIELSEN, E., 1962. Inactivation of the photochemical mechanism in photosynthesis as a means to protect the cells against high light intensities. *Physiol. Plant.*, **5**: 161–171.

STIVEN, A. E., 1965. The relationship between size, budding rate, and growth efficiency in three species of hydra. *Res. Pop. Ecol.*, **7**: 1–15.

WHITNEY, D. D., 1907. Artificial removal of the green bodies of *Hydra viridis. Biol. Bull.*, **13**: 524–537.

ZWEIG, G., J. E. HITT, AND D. H. CHO, 1969. Possible mechanisms of the mode-of-action of quinone-herbicides. Pages 1728–1736, in H. Metzner, Ed., *Progress in photosynthesis research, Volume III.* International Union of Biological Sciences, Tubingen.

Reference: *Biol. Bull.*, **151**: 236–246. (August, 1976)

CIRCADIAN RHYTHMS IN CORALS, PARTICULARLY FUNGIIDAE

BEATRICE M. SWEENEY

Department of Biological Sciences, University of California, Santa Barbara, California 93106

During the day, most of the many species of corals which comprise a tropical reef are contracted, and the tentacles are withdrawn within the horney or calcareous skeleton (Abe, 1939; Yonge, 1940). Feeding takes place at night when the tentacles are expanded. However, there are soft and hard corals in which the tentacles are expanded during the day, and in a few species, the tentacles are always expanded unless they are touched or during reproduction (Wells, 1966). To this author's knowledge, the behavior of corals has not been observed previously under either continuous light or constant darkness in the laboratory. Without such observations, it is impossible to determine whether polyp expansion is directly determined by environmental light or whether it is wholly or partly under the control of a circadian rhythm.

The opportunity for making such observations was provided at the shore station which was established at the Banda Islands, Indonesia by the ALPHA HELIX expedition to Indonesia in the spring of 1975. Species of the scleractinian family Fungiidae are particularly common in the Banda region (Wells, 1966). They are large single polyps not attached to the substrate when mature, and hence they can easily be collected without damage. Fungiids were thus particularly singled out for study. The following is a report of the evidence that tentacle expansion and contraction are controlled both by environmental light and darkness and by a circadian rhythm.

Materials and Methods

Coral specimens were collected from the reefs around the Banda Islands of Neira, Gunnan Api and Banda Bessar while snorkeling. The species studied are listed in Table I. Specimens were placed in a pail under water and then brought to the laboratory without exposure to air. Fungiids were picked up from the bottom, to which they are not attached when mature. Specimens of *Tubipora* and *Euphyllia* were dislodged from a colony with a section of pipe or an abalone iron. On returning to the shore station, specimens were immediately transferred to the experimental tank without exposing them to the air. All specimens were assigned a number on collection. All fungiid specimens used in this study have been deposited in the National Museum, Washington, D. C.

Two experimental tanks of 77 l capacity were filled with unfiltered sea water from the inner harbor at Banda Neira. The water was aerated continuously by a stream of bubbles from an air pump. The temperature of the tanks was 28 ± 0.2° C. The sea water was replaced every four to five days, or sooner if it became cloudy. Zooplankton was occasionally added to the tanks as food, but not according to a regular schedule. One of the experimental aquaria was exposed to natural light from a north-facing window just behind it. Since Banda lies close to the equator at

Table I

Corals observed in aquaria in the laboratory and the presence of circadiam rhythmicity in the expansion and contraction of their tentacles.

Species	Specimen number	Rhythmicity demonstrated in:		
		LD	DD	LL (low 1)
Fungiidae				
Cycloseris laciniosa Boschma	43	+		
Fungia fungites Linnaeus	4	+		
	25		+	
	26	+		
	29	+	+	
	30	+		
	40	+		
Fungia paumotensis Stutchbury	1	+	+	
	2		+	
	15		+	
	28	+		+
	32		+	
	36	+		
	42	+		
Fungia echinata Pallas	37	+	+	
	39	+	+	
Fungia repanda Dana	5	+		
	13	+	+	
	16	+		+
	27	+		
Fungia concinna Verrill	18		+	
Fungia concinna Verrill var.	3	+		
	10		+	
	41	+		
Fungia actiniformis Quoy & Gaimard	11	0		
Polyphyllia talpina Lamarck	7	0		
	8	0		
	35	0		
Halomitra philippinensis Struder	12	+	+?	
Other Corals				
Euphyllia rugosa Dana		0		
Tubipora musica Linnaeus	1	+	?	
	2	+	?	

4.5° S latitude, this tank was brightly illuminated for most of the day. At the time the observations were made, the sun rose at about 0630 and sank at about 1830 local time. The second tank was completely covered with a triple thickness of heavy black plastic sheeting. A corner of this covering was raised for observation of the coral specimens within, which were briefly illuminated by flashlight. Exposure to the flashlight beam did not cause the tentacles to retract if they were expanded.

Coral specimens in both tanks were examined at hourly or two-hourly intervals between 0600 and 2300 local time each day, sometimes also between 2300 and 0600. The time was recorded and the condition of the tentacles was scored as expanded (EX), partially expanded (PC) or contracted (C). It was not practical to measure

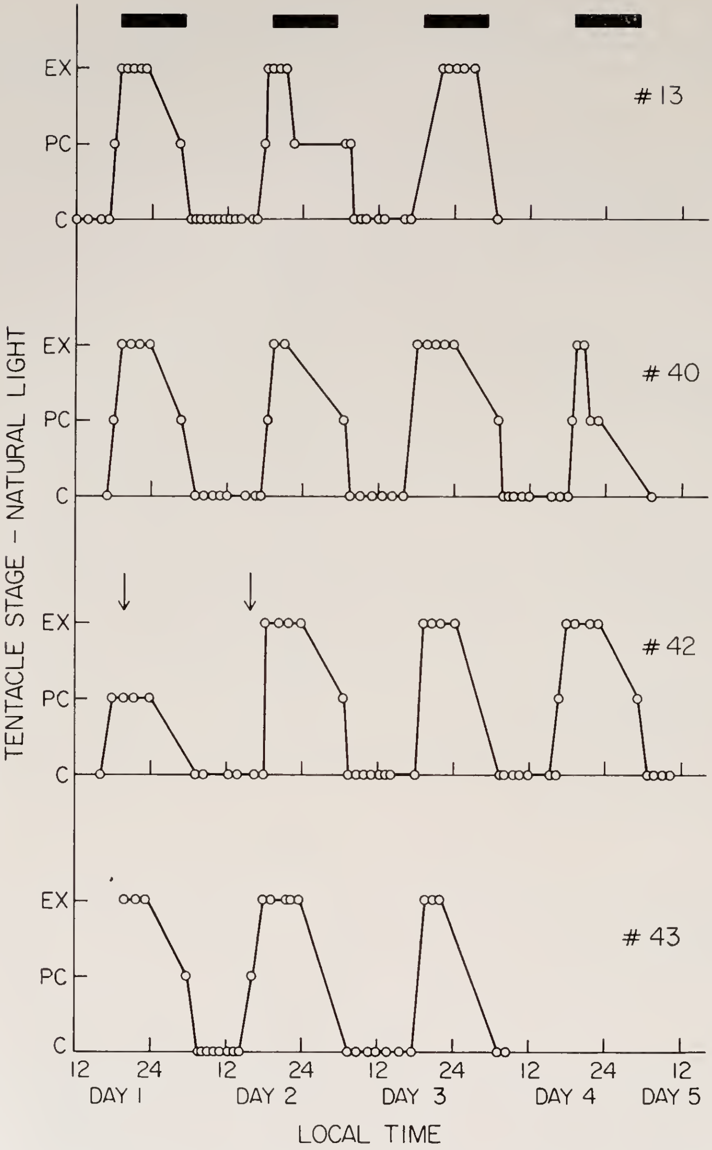


FIGURE 1. The temporal pattern of expansion and contraction of the tentacles in four species of Fungiidae in natural light: No. 13, *Fungia repanda* Dana; No. 40, *Fungia fungites* Linnaeus; No. 42, *Fungia paumotensis* Stutchbury; and No. 43, *Cycloseris laciniosa* Boschma. The tentacle stage was scored as expanded (EX), partially expanded (PC) or contracted (C). Arrows denote the time when zooplankton was added to the aquarium. The dark periods in the environment are shown as black bars on the abscissa. The temperature was kept at 28 ± 0.2° C.

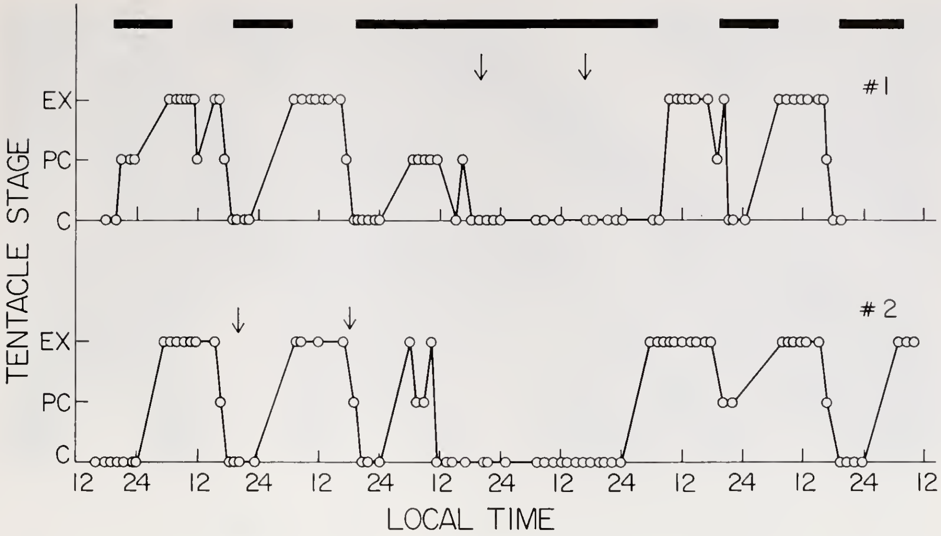


FIGURE 2. The temporal pattern of expansion and contraction of the polyps of two specimens of *Tubipora musica* Linnaeus in natural light, then during 60 hours in darkness, followed by natural light. The tentacle stage was scored as in Figure 1. Arrows denote the time when zooplankton was added to the aquaria. The dark periods are shown as black bars on the abscissa. The temperature was kept at 28 ± 0.2° C.

the length of the tentacles. Usually specimens were first examined for a day or two in the tank exposed to natural illumination and were then transferred during the night to the continuously darkened tank for further observation. A few specimens were observed in continuous light from cool white fluorescent lamps in an incubator at an intensity of 100 lux at 28 ± 0.5° C. Exceptions to this general procedure will be noted. Only specimens in good condition without signs of damage were used. Sand was placed in the tanks for supporting *Tubipora* in an upright position,

In the experiments testing the light sensitivity of *Fungia*, an electronic flash unit (Sun Pac GT3) giving flashes of 1 msec duration, 6000° K color temperature, was fired once. The subsequent behavior of tentacles was observed at intervals of a minute thereafter until the tentacles were completely reexpanded. For photography of tentacles, a Nikon N355 Photomicrographic dark box was attached to the dissecting scope, and the specimens were illuminated with the flash unit described above.

RESULTS

Observations made in the field while snorkeling over the reefs around the Banda Islands during the day confirmed that the majority of both hard and soft corals were in the contracted condition. An enumeration of the species with expanded or contracted tentacles made in the afternoon on two occasions, one at Banda and the other in Weda Bay, both gave an approximate figure of 80% of all species with contracted tentacles. Enumeration at night was attempted from a boat but visibility was insufficient to allow a count. Snorkeling at night in these shark-infested waters is not inviting and was not attempted.

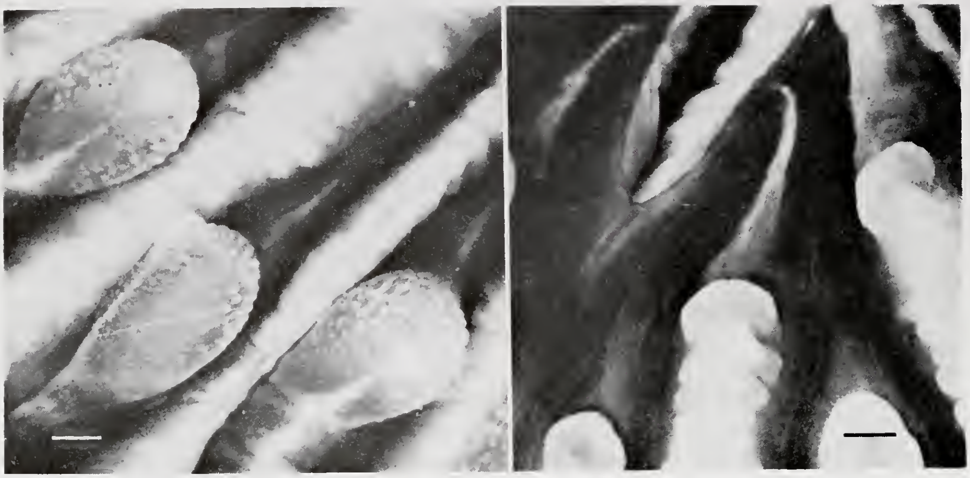


FIGURE 3. Tentacles of *Fungia paumotensis* Stutchbury shown in the expanded (left) and contracted condition (right). The scale bar equals 1 mm.

In the laboratory aquarium under natural illumination, the specimens of Fungiidae behaved in one of two ways (Table I): either the tentacles expanded about one hour after dark and contracted at or somewhat after dawn (Fig. 1), or they remained expanded continuously. Only *Fungia actiniformis* and three specimens of *Polyphyllia talpina* fell into the latter category. One non-fungiid coral, *Euphyllia rugosa*, also remained expanded both day and night under natural light. In none of the specimens of Fungiidae were the tentacles expanded during the day and contracted at night. This behavior was observed in two specimens of *Tubipora musica* (Fig. 2).

Scoring the stage of tentacle expansion was not difficult, since the tentacles of many species of Fungiids are quite long, 3–15 mm, when expanded (Fig. 3). The tentacles of *Fungia actiniformis* are much longer, 50 mm or more. The polyps of *Tubipora musica* showed white against the red skeleton when expanded, while in *Euphyllia rugosa*, the large light-green polyps are easily visible.

The behavior of all the corals in the laboratory under natural light conformed to that observed on the reef. *Fungia actiniformis* was known from previous field studies to remain expanded continuously except during planulation, the only member of the Fungiidae reported to behave in this manner (Wells, 1966). An additional species, *Polyphyllia talpina*, can now be added to this category.

Under the experimental conditions, expanded tentacles of *Fungia* responded to touch by contracting rapidly and were fully reexpanded after 15–20 minutes (Fig. 4). Tentacles did not contract when illuminated for 10–15 minutes with the flashlight used for observations. The tentacles of *Fungia* can respond to light, however. While expanded, three specimens were illuminated by a single flash of high intensity and short duration. All contracted after a minute's latency (Fig. 4) and reexpanded 10–30 minutes later. Contraction of the polyps in bright light can also be inferred from a comparison of tentacle behavior in natural light with that in continuous darkness, as described below.

To determine whether the contraction of the tentacles during the day was the direct result of illumination or might be under the control of a circadian rhythm, specimens were transferred from the aquarium in natural light to the adjacent aquarium darkened with black plastic. The transfer was usually made during the evening to avoid rephasing a rhythm if one were present. Darkening an organism during the light part of a light-dark environmental cycle is known to rephase circadian rhythms in two organisms (Karakashian and Schweiger, 1976), and does so in *Fungia* (Fig. 7). No specimen which had shown alternating contraction and expansion under natural light remained in the expanded state continuously in darkness. Thus light-induced contraction cannot alone be responsible for the contracted state of tentacles during the day. A distinct circadian rhythm was evident in the records of a number of specimens in continuous darkness (Table I and Fig. 5). The period of the rhythm under these conditions appeared to be somewhat shorter than 24 hr, but tentacle behavior proved too erratic to allow accurate determination of period. The tentacles of one specimen of *Fungia paumotensis* (specimen No. 32) behaved arhythmically when first placed in the darkened tank, but later became clearly rhythmic (Fig. 5). Tentacles remained expanded for a considerably longer time in continuous darkness than in natural light (Fig. 5). This observation suggested that light might play a part in determining the temporal pattern of tentacle behavior.

In contrast to the fungiids examined, *Tubipora muscia*, in which the tentacles were expanded only during the day in natural light, showed a single cycle of expansion in continuous darkness, thereafter remaining contracted (Fig. 2). This behavior was not caused by poor condition of the specimens, since they behaved normally on being returned to natural illumination.

Many rhythms have been shown to continue in constant light of low intensity as well as in continuous darkness. Single specimens of *Fungia paumoteunsis* and *F. repanda* were transferred to constant illumination during a day. In bright con-

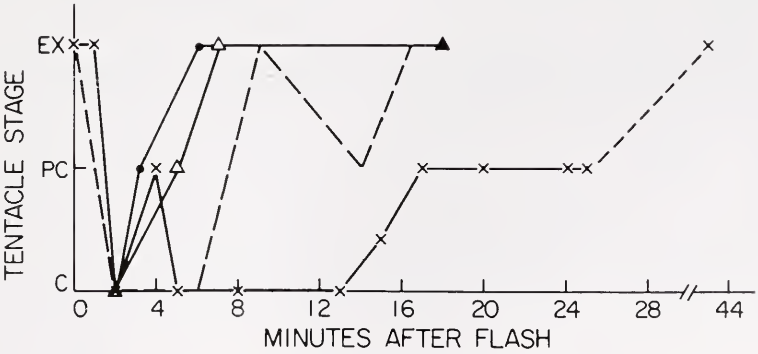


FIGURE 4. The time course of tentacle contraction and expansion following a bright flash, 1 msec in duration, applied at time 0 to specimens with expanded tentacles during the evening in natural light (*Fungia fungites* Linnaeus, open circle; and *F. paumotensis* Stutchbury, open triangle) or in continuous darkness (*F. paumotensis* Stutchbury, x). The dashed line shows the response of *Fungia echinata* Pallas, to touch at time 0. Tentacle stage is scored as in Figure 1.

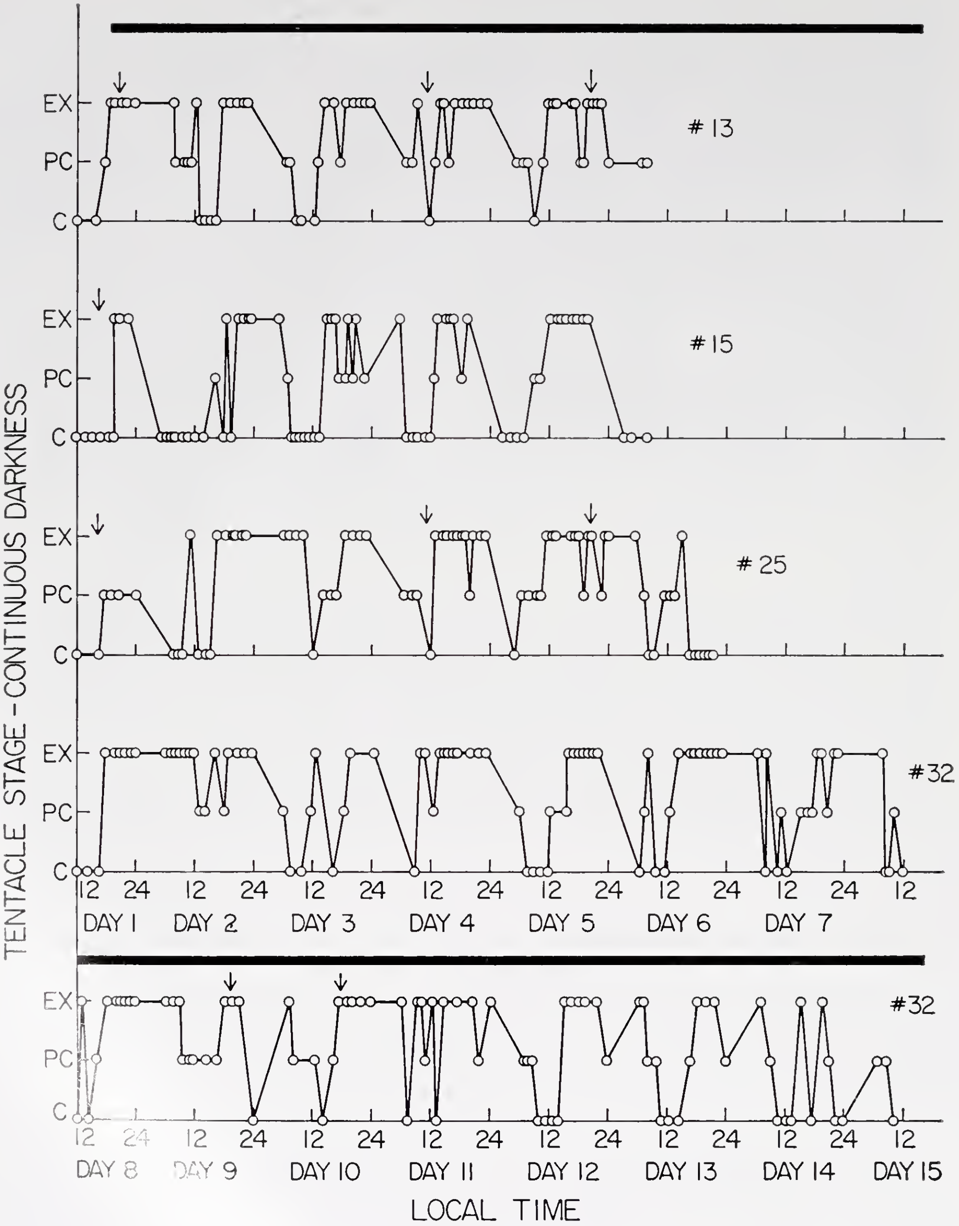


FIGURE 5. The temporal pattern of expansion and contraction of the tentacles in three species of *Fungia* in continuous darkness, as shown by the black bar on the abscissa. No. 13 is *Fungia repanda* Dana; No. 15 and No. 32 are *F. paumotensis* Stutchbury; and No. 25 is *Fungia fungites* Linnaeus. Arrows denote the time when zooplankton was added to the aquarium. Tentacle stage was scored as in Figure 1. The temperature was kept at 28 ± 0.2° C. A circadian rhythm is clearly discernible.

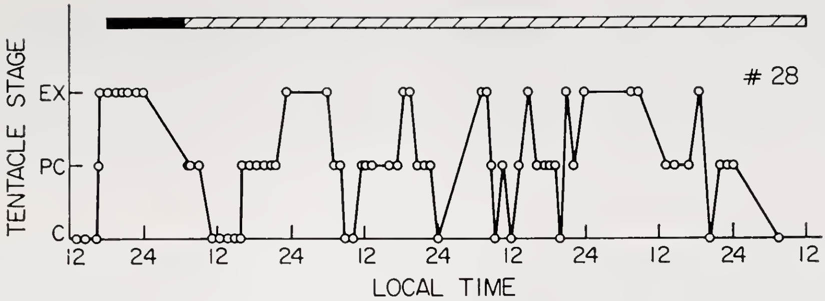


FIGURE 6. The temporal pattern of expansion and contraction of tentacles of *Fungia paumotensis* Stutchbury in natural light for one day, then transferred to continuous light of low intensity (100 lux), as shown by the hatched bar on the abscissa. The temperature was kept at 28 ± 0.5° C. A circadian rhythm is apparent during the first two days in continuous light. Tentacle stage was scored as in Figure 1.

tinuous light, the tentacles remained contracted. However, in light of low intensity (100 lux at 28° C), these specimens displayed a clear circadian rhythm (Fig. 6).

DISCUSSION

The strongly nocturnal behavior of most corals in nature suggests that tentacle expansion might be under the control of a circadian clock, in view of the large number of other nocturnal animals in which such a mechanism has been shown to operate (De Coursey, 1960; Halberg, 1959; Harker, 1954). Among the Coelenterates, however, circadian rhythms have been demonstrated only in the sea-pen, *Cavernularia obesa* (Mori, 1960) and the sea-anemone, *Metridium senile* (Bantham and Pantin, 1950). In both, the rhythmic patterns of body contraction and expansion are somewhat irregular in constant darkness as compared with circadian rhythms in some other organisms under similar conditions, for example in the dinoflagellate *Gonyaulax polyedra* (Sweeney and Hastings, 1957). As in *Fungia*, some specimens of *Metridium* clearly display a circadian rhythm while others do not. In *Metridium* as in *Fungia*, rhythmicity may be obscured at the beginning of exposure to continuous darkness, only to become apparent later in a series of measurements (compare Fig. 5 of this paper with Fig. 8 of Bantham and Pantin, 1950). Since single-polyp Fungiidae are essentially calcified sea anemones, this similarity in behavior is perhaps understandable. In the sea pen, in the sea-anemone and in *Fungia*, strong illumination causes contraction, and hence serves to synchronize behavior with environmental day and night more closely than does the circadian control. No tidal cycles were observed in the corals studied in Banda; however, these corals are never exposed to the atmosphere even at the lowest spring tides.

In corals, the nocturnal activity is correlated with the frequency of zooplankton in the water column. Many more crustaceans were collected in plankton hauls at night than during the day in the Banda region during these experiments, as is generally the case. It is thought that such zooplankton organisms are the principal food of corals (Yonge, 1930, 1968, 1973).

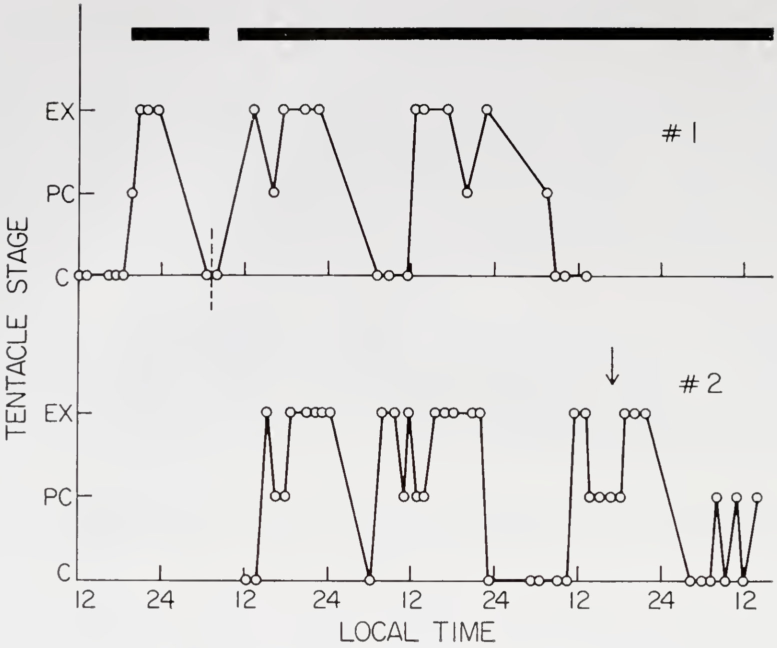


FIGURE 7. The pattern of expansion and contraction of the tentacles of two species of *Fungia* (No. 1, *F. paumotensis* Stutchbury; No. 2, *F. concinna* Verrill var.) exposed to natural light for one day, then transferred to continuous darkness during the day, at 1030 local time, as shown by the black bar on the abscissa. The arrow denotes the time when zooplankton was added to the aquarium. The tentacle stage was scored as in Figure 1. The temperature was kept at 28 ± 0.2° C. The rhythmic expansion of the tentacles in both specimens after the transfer to constant darkness was phase-shifted, so that the tentacles were now expanded during the local day.

Since fungiids can be maintained in a simple aquarium without running sea water and behave normally for long times, they may become useful experimental animals. They also have the advantage of large size, simplicity of organization and ease of collection. It should prove interesting to analyze their circadian rhythm further. Tentacle expansion is thought to be mediated by contraction of the body muscles which forces water into the tentacles. This contraction must be accomplished by activity in the nerve net. Such activity has been measured directly in *Renilla kollikeri* by insertion of microelectrodes (Anderson and Case, 1975). It is possible that a circadian rhythm in the firing of the nerve net could be detected in *Fungia*. A circadian rhythm in nerve impulses has been documented in the parabolic burster cell of the parieto-visceral ganglion (Strumwasser, 1965) and in the optic nerve (Eskin, 1972) of the sea hare *Aplysia californica*. Such studies might thus be particularly pertinent in analyzing the nature of a circadian clock, in view of the current theories of membrane involvement in this mechanism (Njus, Sulzman and Hastings, 1974; Sweeney, 1974).

The author wishes to express her great appreciation to Professor John H. Wells for identifying all the fungiids used in this study. This study of the ALPHA HELIX East Asian Bioluminescence Expedition was supported by the National Science Foundation under grants OFS 74-01830 and OFS 74-02888 to the Scripps Institution of Oceanography and NSF grant BMS 74-23242 to the University of California, Santa Barbara.

SUMMARY

The temporal patterns of expansion and contraction of the tentacles of seven species of *Fungia*, three other species of the family Fungiidae and two other scleractinian corals have been examined in the laboratory. In six species of *Fungia*, tentacle behavior showed a circadian rhythm, both under natural light and in continuous darkness, while in two species a circadian rhythm was also demonstrated in continuous light of low intensity. *Fungia actiniformis, Polyphyllia talpina* and *Euphyllia rugosa* remained expanded under all conditions. The phase of the tentacle rhythm in *Fungia* could be changed by initiating continuous darkness during the day. Tentacles of *Fungia* were shown to contract in strong illumination. Contraction could be induced by a bright flash of 1 msec duration, after a latency of about one minute. The significance of these findings is discussed.

LITERATURE CITED

ABE, N., 1939. On the expansion and contraction of the polyp of a reef-coral, *Caulastrea furcata. Dana. Palao Trop. Biol. Sta. Studies I,* **4**: 651–670.

ANDERSON, P. A. V., AND J. F. CASE, 1975. Electrical activity associated with luminescence and other colonial behavior in the pennatulid *Renilla kollikeri. Biol. Bull.,* **149**: 80–95.

BANTHAM, E. J., AND C. F. A. PANTIN, 1950. Phases of activity in the sea-anemone, *Metridium senile* (L.), and their relation to external stimuli. *J. Exp. Biol.,* **27**: 377–399.

DE COURSEY, P. J., 1960. Phase control of activity in a rodent. *Cold Spring Harbor Symp. Quant. Biol.,* **25**: 49–55.

ESKIN, A., 1972. Phase shifting a circadian rhythm in the eye of *Aplysia* by high potassium pulses. *J. Comp. Physiol.,* **80**: 353–376.

HARKER, J. E., 1954. Diurnal rhythm in *Periplaneta americana* L. *Nature,* **173**: 689–690.

HALBERG, F., 1959. Physiologic 24-hour periodicity in human beings and mice, the lighting regimen and daily routine. Pages 803–878 in R. B. Withron, Ed., *Photoperiodic and related processes in plants and animals.* American Association for the Advancement of Science, Washington, D. C.

KARAKASHIAN, M. W., AND H. G. SCHWEIGER, 1976. Circadian properties of the rhythmic system in individual nucleated and enucleated cells of *Acetabularia mediterranea. Exp. Cell Res.,* in press.

MORI, S., 1960. Influence of environmental and physiological functions on the daily rhythmic activity of a sea-pen. *Cold Spring Harbor Symp. Quant. Biol.,* **25**: 333–344.

NJUS, D., F. M. SULZMAN, AND J. W. HASTINGS, 1974. Membrane model for the circadian clock. *Nature,* **248**: 116–120.

STRUMWASSER, F., 1965. The demonstration and manipulation of a circadian rhythm in a single neuron. Pages 442–462 in J. Aschoff, Ed., *Circadian clocks.* North-Holland Publishing Co., Amsterdam.

SWEENEY, B. M., 1974. A physiological model for circadian rhythms derived from the *Acetabularia* rhythm paradoxes. *Int. J. Chronobiol.,* **2**: 25–33.

SWEENEY, B. M., AND J. W. HASTINGS, 1957. Characteristics of the diurnal rhythm of luminescence in *Gonyaulax polyedra. J. Cell Comp. Physiol.,* **49**: 115–128.

WELLS, J. W., 1966. Evolutionary development in the scleractinian family Fungiidae. Pages

223–246 in W. J. Rees, Ed., *The Cnidaria and their evolution.* Symposia of the Zoological Society of London, No. 16. Academic Press, London.

YONGE, C. M., 1930. Studies on the physiology of corals. I. Feeding mechanisms and food. *Brit. Mus. (Natur. Hist.) Great Barrier Reef Exped. 1928–1929,* **1**: 13–57.

YONGE, C. M., 1940. The biology of reef-building corals. *Brit. Mus. (Natur. Hist.) Great Barrier Reef Exped. 1928–1929,* **1**: 353–391.

YONGE, C. M., 1968. Living corals. *Proc. Roy. Soc. London Ser. B,* **169**: 327–344.

YONGE, C. M., 1973. The nature of reef-building (hermatypic) corals. *Bull. Mar. Sci.,* **23**: 1–15.

Reference: *Biol. Bull.*, **151**: 247–259. (August, 1976)

SAND DOLLARS AS SUSPENSION FEEDERS: A NEW DESCRIPTION OF FEEDING IN *DENDRASTER EXCENTRICUS*

PATRICIA L. TIMKO[1]

Department of Biology, University of California, Los Angeles, California 90024

This paper describes the feeding behavior, diet, and food size preference of the common Pacific sand dollar, *Dendraster excentricus*. *Dendraster excentricus* is abundant along the Pacific coast of North America from Juneau, Alaska, to central Baja California (Wagner, 1974). This study concentrates on *Dendraster* from Southern California, where the sand dollars inhabit subtidal surf-swept beaches, quiet bays, and estuaries (Merrill and Hobson, 1970).

Individuals of *Dendraster* feed in an inclined posture (Fig. 1) with the anterior portion of the test inserted into the sand when a slight to moderately fast current is running (Chia, 1969a; Merrill and Hobson, 1970). Previous descriptions of feeding in *Dendraster* dealt only with the entrapment of small particles in ciliary currents generated by the epithelium of the spines (MacGinitie and MacGinitie, 1949; Chia, 1969a). In the past, sand dollars have generally been regarded as deposit or detrital feeders (Reese, 1966). However, the present work reports methods of capture of large particles (>50 μm) and small active prey. Specimens of *Dendraster* from Puget Sound have been reported to eat diatoms, algae, and sand grains (Chia, 1969a), but no quantitative analysis of their diet or selectivity has been described to date. This dearth of information is remarkable in view of the great abundance and extensive geographic range of *Dendraster excentricus*.

MATERIALS AND METHODS

Specimens of *Dendraster excentricus* from a sub-tidal, protected outer coast population at Zuma Beach, Los Angeles County, California, were used except as noted. Laboratory specimens were kept in holding tanks connected to a 1500 gallon recirculating seawater system; they consumed detritus in the tanks as well as the *Artemia salina* nauplii provided as food. All experiments with live specimens of *Dendraster* were done at 12 to 14° C (normal temperature range at Zuma Beach).

Feeding behavior in the inclined position was observed with a dissecting microscope mounted on a boom arm (Bausch and Lomb). Food was placed on the test surface with a taper cut catheter tube connected to a syringe barrel. Large food items (*Artemia*, Sephadex beads, sand grains, etc.) were inserted into the food grooves by the same method. Diatom suspensions (*Navicula distans*) were introduced in a #21 syringe needle connected to a catheter.

Sand dollars were force-fed by gently inserting a syringe needle or catheter tube beneath the buccal spines and injecting the food. Great care was necessary to avoid touching the spines or the peristomal membrane, since the sand dollars would reject the food if disturbed in this manner.

---

[1] Present address: Department of Zoology, University of Wisconsin, Madison, Wisconsin 53706.

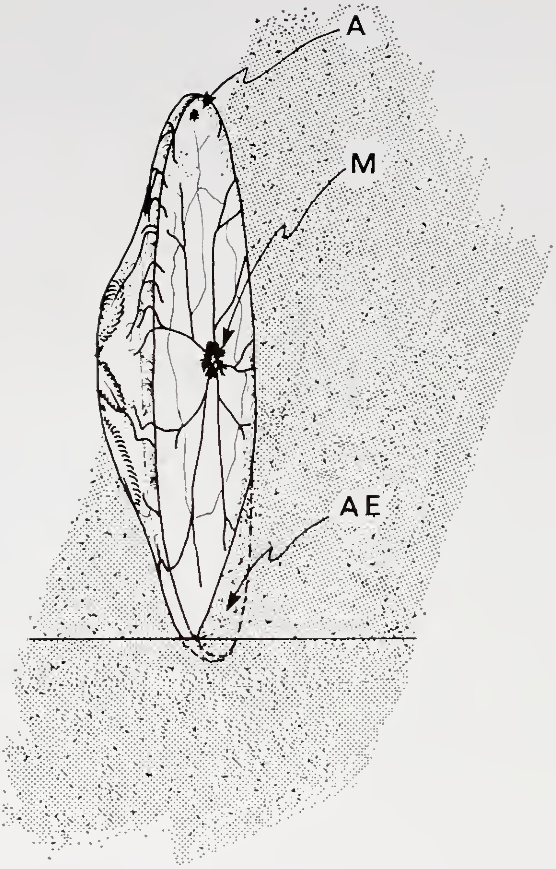


FIGURE 1. Inclined posture of *Dendraster excentricus*. The anterior edge of the test (AE) is inserted into the sand. In this position, sand dollars suspension feed by capturing particles which come in contact with the test. The mouth is indicated by M, the anus by A.

For diet analysis, animals collected in the field were immediately fixed in 80% ethanol after being brought from the water. The sand dollars were dissected and the bolus of food nearest the mouth was removed. Each bolus was divided into 3 or 4 equal parts and each part was smeared on a glass slide, dried, and stained with 1% Nile Blue Sulfate or Lugol's Iodine. Chitin was detected by fluorescence microscopy (Leitz fluorescence microscope). Five fields of view at 100× magnification (which covered about 30% of each smear) were selected at random on each slide; each item therein was identified and its surface area was measured with an eyepiece micrometer.

For quantitative measurements of gut contents, the tissues were allowed to harden in 80% ethanol for one week, after which the gut was excised intact. The gut was slit open and the contents were removed, washed with distilled water, dried, and weighed. The gut contents were then hydrolyzed in hot concentrated chromic acid for 48 hr, washed three times in distilled water, dried, and reweighed. The amount of organic matter in the gut contents was approximated as the loss in dry weight following acid hydrolysis.

Captured material held in the spines and tube feet was collected by injecting the sand dollars intraperistomially with 0.5 ml 0.54 M KCl solution immediately after the animals were brought from the water. After about 30 sec, the sand dollars would release anything that the spines and tube feet held, and the material was collected in a dish.

For the experimental determination of size selectivity, a sand dollar was positioned in the inclined posture by inserting the anterior end into a block of agar; the animal was placed in a 15 liter tank in which a unidirectional water flow was maintained at 11 cm/sec (a normal current speed for Zuma Beach; Timko, 1975). Equal numbers of fluorescent plastic beads (Duke Standards, Palo Alto, California) of 30, 40, 50, 80, and 100 μm diameters were added to the water, giving a total of 0.1 g beads in the tank. After one hr in the tank, the sand dollar was removed, fixed in 80% ethanol, and the beads it had captured on its oral surface were scraped off. The beads were counted and their sizes measured on a Leitz fluorescence microscope.

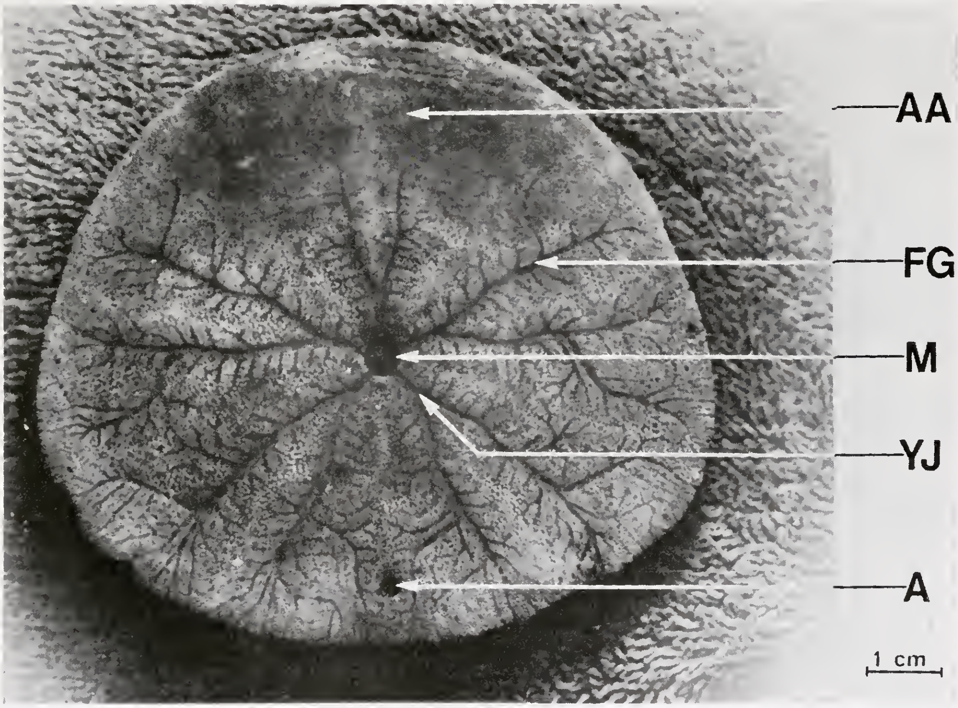


FIGURE 2. Oral surface of a *Dendraster* test that has been denuded of spines. The anterior portion of the test which is inserted into the sand is marked AA. One of the Y junctions, the major intersections of the food grooves (FG) near the mouth (M), is marked by YJ. The anus is indicated by A. The maximum diameter of the sand dollar in the photo is 84 mm.

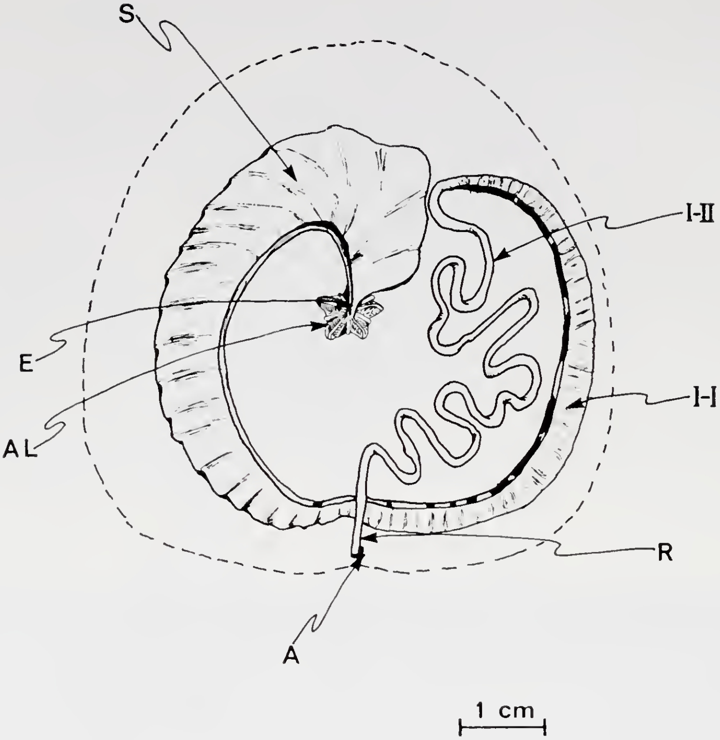


Figure 3. Digestive organs of *Dendraster*, viewed from the aboral side. The outline of the test is marked by a dashed line. Aristotle's Lantern (AL) is the masticating apparatus. Esophagus (E), stomach (S), intestine I (I-I), intestine II (I-II), and rectum (R) follow the terminology of Reisman (1965). The anus (A) exits on the oral surface. In an average size sand dollar (maximum length about 60 mm), the length of the different gut sections would be: esophagus, 5 mm; stomach, 70 mm; intestine I, 70 mm, intestine II, 90 mm; rectum, 10 mm.

Results

Brief description of feeding structures and digestive system

Specimens of *Dendraster* were observed feeding in the inclined posture (Fig. 1) and the prone posture, in which they lay flat upon the substrate or buried in it. Feeding behavior in either position was identical, except as noted below.

The oral surface of *Dendraster* is covered by straight primary spines about 4 mm long, secondary spines about 1 mm long, numerous suckered tube feet, and bidentate pedicellariae. The pedicellariae are of two size classes, the larger averaging 0.6 mm long and the smaller averaging 0.14 mm long (Chia, 1969b). Food grooves (Fig. 2), lined with stubby, non-suckered ambulacral tube feet, extend over the oral surface except for the area which is usually inserted into the sand in the inclined posture. Some food grooves extend over the margins and onto the aboral surface for a short distance.

The mouth is contained within a buccal cavity that is covered by five groups of straight buccal spines which are about 4 mm long. The floor of the buccal cavity is formed by the peristomal membrane, which overlays Aristotle's lantern. Buccal tube feet, which are similar to ambulacral tube feet but slightly longer, line the area where the five main food grooves enter the buccal cavity.

The digestive tract has five distinct regions (Fig. 3): esophagus, stomach, intestine I, intestine II, and rectum (Reisman, 1965). The digestive organs are suspended by mesentaries within the central cavity of the test.

Food capture

Three types of food handling behavior were observed which depended upon whether the food was motile prey, nonmotile material, or particles <50 μm in diameter.

Dendraster is capable of capturing actively swimming prey. Laboratory observations showed *Dendraster* easily caught and ingested about 80% of the small crustaceans (nauplii of *Artemia salina*, mysids, calanoid copepods, etc.), that contacted the oral surface. The spines, tube feet, and large bidentate pedicellariae were used in prey capture. Prey capture was observed only on the oral surface of the sand dollar. Initially, when prey contacted the oral surface, the primary spines within about 1 mm of the contact moved their distal ends together, forming a cone-like trap over the prey (trapping response). When a sand dollar was actively capturing prey, as when given a meal of *Artemia*, the numerous cone traps were apparent to the unaided eye. Within a few seconds of the trapping response, the large bidentate pedicellariae extended with open jaws. If prey were nearby, the jaws snapped open and shut vigorously, usually resulting in the rapid capture of the prey. The prey rarely escaped from the pedicellariae; furthermore, the cone traps hampered the prey from swimming away. In flowing water, the cone traps may also keep prey from being swept off the test by the current.

After snapping on the prey a few times, the pedicellariae released it and the spines and tube feet moved it toward a food groove. The tube feet generally pushed the prey, whereas the spines would strike or bat it. Along the route to the food groove, several other pedicellariae often snapped on the prey. This process of "pre-oral mastication" resulted in a food particle that was already somewhat macerated when it reached the food groove. Upon reaching the margin of a food groove, the prey mass was transported into the groove by the tube feet which are densely distributed along the margin. Several tube feet pushed the mass into the groove or a single tube foot grasped the mass and placed it into the groove.

Nonmotile food items >50 μm in diameter (sand grains, algal fragments, etc.) were grasped by the tube feet and pushed toward a food groove. The pedicellariae did not assist in handling nonmotile foods, unless the food was quite large. Ciliary currents were too weak to move large food items effectively.

Ciliary currents were used in the transport of particles <50 μm in size. The small particles traveled very closely to the test surface and were swept into the food grooves by the ciliary currents. These currents were generated by the cilia along the base of the spines (Chia, 1969a).

Transport in the food groove

Small particles were enveloped in the mucus secreted in the food groove. The stubby ambulacral tube feet passed the mucus strings toward the mouth. Larger

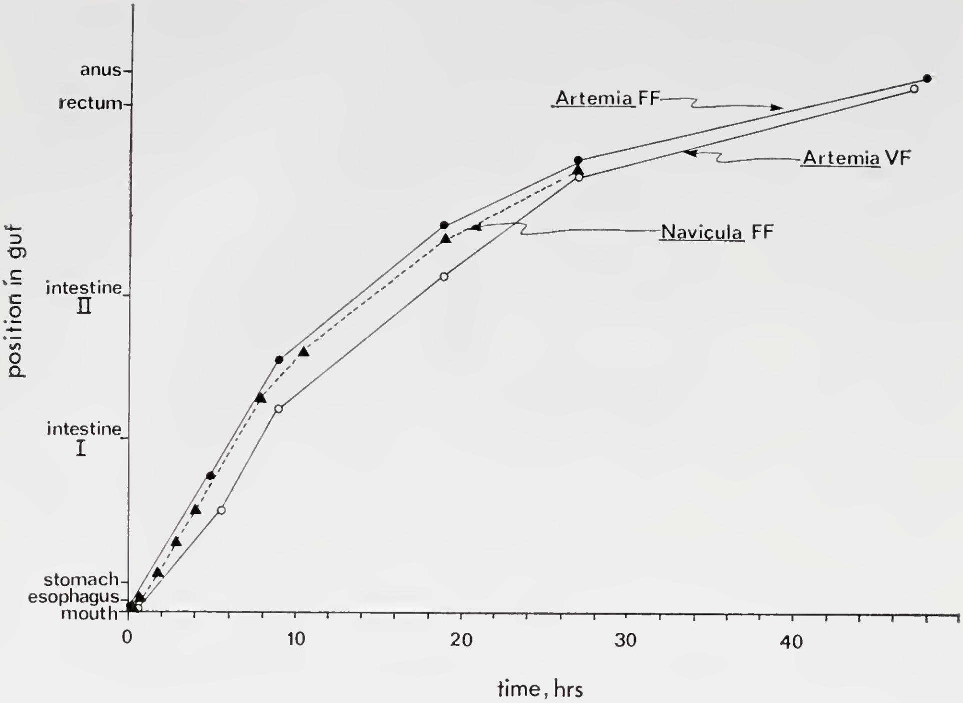


FIGURE 4. Clearance rate of food through the *Dendraster* gut. The ordinate shows the position of the food in the gut and represents linear distance (actual distance varied with the size of the animal; see Fig. 3). Solid circles represent the rate of passage for a meal of *Artemia* force fed to the sand dollars, open circles represent the rate for a meal of *Artemia* fed free choice to the sand dollars, and the solid triangles represent the rate for a meal of *Navicula* force fed to the sand dollars.

items, such as algal filaments and single crustacean prey, were moved without visible evidence of a mucus string. If the food item was several mm long (*e.g.*, pieces of *Phyllospadix* (surf grass) or small polychaetes), it was moved by a coordinated rowing motion of the ambulacral tube feet in contact with the food. The period of a stroke and retraction was about two sec.

When food reached the buccal cavity, it was drawn in by the buccal tube feet. Small items could be taken in by ciliary currents. In the case of foods several millimeters long, the buccal spines (which normally lay flat over the buccal cavity) were raised to admit the large item. Food was drawn past the peristomal membrane by the teeth, which thoroughly ground the food prior to swallowing.

The time for the entire sequence from capture to ingestion varied from 5 to 30 min, with active prey requiring more time than nonmotile items. The average time from prey capture to ingestion was about 15 min, and the teeth usually masticated the prey for another 15 min prior to swallowing.

Rejection response

Selective rejection of food occurred at two sites: the test surface and the Y junction of food grooves near the mouth (Fig. 2). If an item which *Dendraster* would not eat (Sephadex beads, bits of agar, acid cleaned sand grains) was placed onto the oral surface, the spines waved vigorously and the material was moved away from the food grooves and toward the nearest test edge, where it was dropped. Often, when the sand dollar was feeding in the prone position, rejected items were pushed only for a short distance, then dropped onto the substrate. The small pedicellariae frequently emerged during the rejection response, but the large pedicellariae did not.

Items which were rejected were also tested for acceptance by injection into a food groove. At times, the injected material was not moved at all. Most often, however, the material was moved toward the mouth, but upon reaching the Y junction, was pushed out of the food groove by the ambulacral tube feet. The Y junction appeared to be the final sorting point for food prior to ingestion, since Sephadex beads that were injected beyond the Y junction at the edge of the buccal cavity were ingested.

Occasionally, a *Dendraster* regurgitated gut contents if the chamber water was not kept cool or sufficiently aerated. During regurgitation, the buccal spines were raised, the gut contents were ejected through the mouth, and the rejection response ensued. In addition, the spines bordering the food grooves interlaced over the food grooves preventing the regurgitated material from entering.

Defecation

Unlike the sand dollar *Mellita sexiesperforata*, in which the anus is near the mouth, and which ceases feeding during defecation (Goodbody, 1960), individuals of *Dendraster* continued to feed during defecation. Immediately prior to defecation, the periproct was elevated. At times, the periproct would open and water would be taken in and expelled three or four times (anal irrigation) before defecation. Anal irrigation was not a prerequisite for defecation. Feces were ejected in a flocculent jet which extended about 5 mm from the test when the sand dollar was in the inclined posture. If the sand dollar was prone, the feces were expelled while the animal crawled about, leaving a trail of feces behind. During defecation, the marginal spines interlaced over the food groove which lays between the mouth and anus, preventing reingestion of feces. If any fecal material touched the test surface, the rejection response was observed in that area.

Clearance rate and feeding times

The rate of food passage through different sections of the gut of normally feeding sand dollars was examined by feeding the sand dollars nauplii of *Artemia* vitally stained with 1% Nile Blue Sulfate. Ten sand dollars were placed in a small tank and offered the stained *Artemia* nauplii free choice (voluntarily fed group) for 1 hr, after which the sand dollars were returned to their normal holding tanks. Ten other sand dollars were force-fed by injecting stained *Artemia* nauplii into the buccal cavity. The force-fed animals were kept in small dishes for 1 hr, then returned to their holding tanks. In the holding tanks, both groups of sand dollars resumed their normal ingestion of sand and detritus. At intervals from 6 to 48 hrs following the meal of *Artemia*, two sand dollars from each group were sacrificed, their guts examined, and the position of the stained nauplii marked (Fig. 4).

It took slightly under five hours for the marked food to pass through the stomach and about 10 hours for it to pass through intestine I (Fig. 4). After two days, the labelled food passed the rectum and reached the anus. The rate of progress was similar in voluntarily-fed and force-fed groups, but the absolute position of the food in voluntarily-fed sand dollars lagged about 0.5 hr behind that of force-fed sand dollars. The lag represents the time required for capture and ingestion of the *Artemia* nauplii.

The rate of passage was also measured by using a suspension of *Navicula distans* (a diatom) to account for possible differences in digestive rate for another type of food. Since specimens of *Navicula* were difficult to distinguish from other gut contents, only starved, force-fed sand dollars were used in the experiment. The rate of passage for the meal of *Navicula* corresponded closely to that of the meal of *Artemia* (forced-fed group). Therefore, the rate of passage through the gut was not a function of the type of food ingested.

Determination of the rate of passage of the food allowed estimation of feeding times in the field by extrapolating from the position of the food in the gut. Ten sand dollars were collected from the population at Zuma Beach at 10:00 (twice), 12:00, and 15:40 on different days. The animals were fixed immediately after being brought from the water.

The data indicate that individuals of *Dendraster* fed continuously with occasional pauses. There were few consistent trends in the distribution of food in the guts. In all samples, 80% of the sand dollars were actively feeding (food in the teeth or buccal cavity) at the time of collection. Material in the stomach was in discrete boluses until it reached intestine I, after which it was well packed with few gaps. The gaps between boluses in the stomach indicated that intervals of 15 to 30 min separated the swallowing of each bolus, which is consistent with laboratory observations. Gaps in food distribution in the intestines occurred in 28% of the animals, denoting lapses of 1 or 2 hr duration; the reason for the pauses is unknown.

Diet

The diet of *Dendraster* from Zuma Beach was determined by microscopic examination of smears of gut contents. Since the food was well masticated before ingestion, it was not possible to identify the numbers of whole prey or other food items that had been eaten. Instead, the surface area of each food fragment was measured, since the gut smears were essentially two dimensional. The food bolus nearest the mouth, divided into 3 to 4 smears, was examined from ten animals on each date.

The seasonal composition of the diet varied considerably (Table I). The gut contents in the summer sample were predominated by dinoflagellates (*Gonyaulax polyedra, Ceratium* spp., *Dinophysis homunculus, Noctiluca scintillans*), sand grains, organic detritus (stained material not identifiable), chitin fragments (from decapod zoea, cirripede nauplii, amphipods), and algal fragments. The composition of the gut contents was comparable to the material suspended above the sand dollar bed except that the gut contents had a smaller proportion of sand grains. The sediment from a water sample taken above the sand dollar bed in June contained approximately 30% sand grains, 30% dinoflagellates (mostly *G. polyedra, Ceratium*

Table I

Seasonal changes in diet.

Category	(Feb. 9, 1975) Winter diet, percentage	(June 10, 1974) Summer diet, percentage	(April 16, 1974) During spring plankton bloom, percentage	(March 30, 1974) Two weeks prior to plankton bloom percentage
Centric diatoms	6.2	5.8	90.3	5.5
Pennate diatoms	1.3	1.4	1.0	3.3
Dinoflagellates	1.0	42.0	1.3	2.3
Small flagellates	0.4	0.5	0.3	0.0
Tintinnids	10.6	3.3	0.0	0.5
Radiolaria	5.4	0.6	0.2	2.9
Foramanifera	4.0	0.6	0.0	0.0
Silicoflagellates	1.4	0.2	0.0	0.1
Chitin fragments	41.6	9.6	2.7	23.2
Algal filaments	8.0	6.1	1.0	19.7
Phyllospadix	2.1	0.0	0.0	2.3
Sand grains	13.2	13.9	2.7	12.9
Wood ash	4.0	0.4	0.3	16.0
Echinoid calcite	0.0	1.9	0.0	0.0
Detritus	0.9	13.7	0.0	0.0
Other	0.1	0.0	0.0	0.0

spp., and *D. homunculus*), 20% chitin containing Crustacea (decapod zoea, cirripede nauplii), 10% centric diatoms (three species of *Chaetoceros* and *Coscinodiscus oculus*), 5% algal fragments, and 5% tintinnids (mostly *Parundella minor*). In contrast to the summer gut sample, the most abundant items in the winter gut sample were chitin fragments (from mysids, amphipods, calanoid copepods), sand grains, tintinnids (predominately *P. minor*), algal fragments, and centric diatoms (*C. oculus, C. perforatus, Navicula distans, Biddulphia* (*rhombus*?), *Nitzschia pacifica, Pleurosigma* spp.). Comparing the diet before and after a plankton bloom strengthens the contention that *Dendraster* fed on whatever was abundant and available in the plankton (Table I). Prior to the bloom, the sand dollars were feeding primarily on crustaceans and algal fragments. During the bloom, when the diatom *Chaetoceros* composed more than 90% of the suspended material in the water, the sand dollar diet shifted heavily toward *Chaetoceros*.

The diet of the sand dollars from Zuma Beach was compared with that of sand dollars inhabiting two bays (Morro Bay and Newport Harbor, California) for the amount of food in the gut and food quality. Morro Bay sand dollars had been collected in January, 1973, and Newport Harbor sand dollars had been collected in August, 1973. The Zuma Beach sand dollars which were compared to the Morro Bay sample were collected in January, 1973, and the Zuma Beach sand dollars which were compared to the Newport Harbor sample were collected in August, 1973 (N equals 10 for each sample). Due to differences in body weight among individuals, the weight of food was expressed as a percentage of the wet body weight (food index). Differences between food indices and the organic content of the food were tested with one way ANOVA (Sokal and Rohlf, 1969).

Sand dollars from the protected outer coast population at Zuma Beach had more food of better quality in their guts than sand dollars from the bay populations

TABLE II

Amount and quality of food in the guts of sand dollars from different locations.

Sample	Mean weight of food, gm	Mean food index	Mean relative measure of organic content
Zuma Beach, summer	0.055	0.281	34.7%
Zuma Beach, winter	0.060	0.259	56.6%
Morro Bay, winter	0.023	0.192	45.1%
Newport Harbor, summer	0.021	0.153	20.8%

(Table II). The food index of the Morro Bay sample was lower than that of the January Zuma Beach sample ($P < 0.05$), as was the Morro Bay sample organic content ($P < 0.05$). Similarly, the Newport Harbor sample had a lower food index ($P < 0.05$) and organic content ($P < 0.10$) than the Zuma Beach August sample. The food index did not differ seasonally among the two Zuma Beach samples ($P > 0.10$), but the winter sample contained significantly more organic matter ($P < 0.01$).

Size selectivity

Analysis of the diet indicated that *Dendraster* was relatively nonselective with respect to types of food; in addition, selectivity concerning food size was examined. The particles which had been held in the spines and tube feet of 25 specimens of *Dendraster* from Zuma Beach were analyzed for size by seiving through a U. S. Bureau of Standards seive series (Fig. 5). The sand dollars had captured relatively small particles, 60% being <180 μm. Comparisons with the material suspended above the sand dollar bed were not made due to the small amount of sediment collected in the water samples.

An Ivlev index (Ivlev, 1961) was computed on the basis of laboratory experiments which used fluorescent plastic beads of five different sizes, from 30 μm to 100 μm in diameter. P_i was corrected for passive settling of the beads by comparison with controls containing no sand dollars. Five trials were used to calculate an average electivity index (E) for each particle size.

E ranges from +0.67 for absolute preference to −1.00 for total avoidance of any of the particle sizes. The specimens of *Dendraster* did not strongly prefer or avoid any of the particle sizes (Table III). Therefore, the Ivlev index and the size of the captured particles indicate that *Dendraster* was not selective with respect to food particle size within the range of 1 μm to 180 μm.

DISCUSSION

The feeding habits of *Dendraster excentricus* differ significantly from those of other sand dollars. Unlike *Mellita sexiesperforata* (Goodbody, 1960), *M. quinquesperforata* (Hyman, 1958), and *Echinarachnius parma* (Sokolova and Kusnetzov, 1960), all of which are microphagous deposit feeders, specimens of *Dendraster* consume large particles, capture active prey, and are capable of both prone deposit feeding and inclined suspension feeding. Feeding in *Dendraster* was previously described by both MacGinitie and MacGinitie (1949) and Chia (1969a). Both

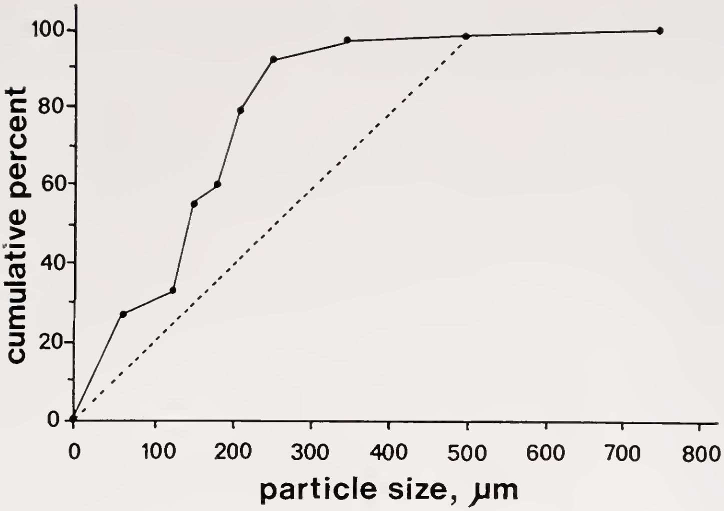


FIGURE 5. Size of captured particles held by the tube feet and spines. If all particle sizes were equally abundant in the sediment collected within the range of 0 to 500 μm, the cumulative percentage of particle sizes would be given by the dashed line. The solid line represents the cumulative percentage for the particle sizes held by the sand dollars. The slope of the solid lines shows that particles of 1 to 225 μm were relatively more abundant ("over-represented") than were particles >225 μm, but the differences in abundance are not striking.

studies reported ciliary mucus feeding, and Chia's paper was the first to describe the role of the ambulacral tube feet in the transport of mucus strings. However, no previous study included observations of feeding in the inclined posture. The present study indicates that the use of spines and suckered tube feet on the oral surface is probably the dominant method of food gathering, rather than ciliary mucus feeding. Most of the particles in the gut were too large to have been moved by the feeble ciliary currents of the oral surface. Furthermore, most large particles were transported in the food grooves without the secretion of much mucus. Therefore, I suggest that *Dendraster excentricus* be considered as primarily a suspension feeder rather than a deposit feeder or ciliary mucus feeder.

TABLE III

Electivity index for different particle sizes. The electivity index for the ith particle size, E_i, *is calculated as* $E_i = (p_i - P_i) \div (p_i + P_i)$, *where* P_i *is the proportion of the ith particle size in the mixture offered to the animal and* p_i *is the proportion of the ith particle size which the animal captures or ingests relative to the other sized particles ingested.*

Particle size, microns	E_i, mean of 5 trials
30	−0.037
40	+0.167
50	−0.141
80	+0.061
100	−0.195

Inclined individuals of *Dendraster*, especially in dense aggregations which hydrodynamically enhance the efficiency of particle capture (Timko, 1975 and in preparation) are extremely effective suspension feeders. The fact that *Dendraster* captured and ate active prey has consequences when the community structure of sandy bottom areas is considered. In areas where sand dollars form dense beds, such as in Southern California (Merrill and Hobson, 1970), the sand dollars are probably important benthic suspension-feeding predators which consume large numbers of small prey such as mysids, amphipods, copepods, and the larvae of other benthic animals which attempt to settle in the area.

Chia (1969a) reported that specimens of *Dendraster* which he examined from Puget Sound, Washington, invariably had empty stomachs. Specimens of *Dendraster* examined in the present study usually had food in the stomach, but the boluses were spaced since the sand dollars masticated a mouthful of food for about 15 minutes before swallowing it. These food boluses passed out of the stomach fairly rapidly (in 5 hr). Although other echinoids are known to exhibit diurnal periodicity in feeding (Lawrence and Hughes-Games, 1972), *Dendraster* was found to feed continuously in this study. This result is to be expected, since there is no known diurnal variation in food availability nor is there any possibility of evading visually-hunting predators in the daytime.

Reports of diet composition and selectivity in sand dollars are scarce. Hyman (1958) stated that the gut of *M. quinquesperforata* contained nannoplankton but no sand grains. *M. sexiesperforata* specialized on particles <20 μm in size (Goodbody, 1960). Chia (1969a) recovered diatoms, sand grains, and pieces of algae from the food grooves of Puget Sound *Dendraster* and suggested that the diet was generalized. The data on size selectivity and diet presented here confirm the generalized nature of the diet of *Dendraster*. The instances of selective rejection indicated that a criterion other than size must be the basis for rejection. The role of the tube feet in rejection is especially interesting, since rejection appears to be initiated by the tube feet on the oral surface and those at the Y junction.

Chia (1969a) hypothesized that a generalized diet might contribute to the abundance of *Dendraster*. In addition to a generalized diet, the efficient prey-handling behavior and continuous feeding reported here are undoubtedly important factors which have allowed *Dendraster excentricus* to attain great abundance and widespread distribution.

I would like to thank Dr. James G. Morin and Dr. Jon E. Kastendiek for collecting the subtidal samples of Zuma Beach sand dollars. Dr. Morin assisted throughout the completion of this project. This paper is taken from research done in partial fulfillment of the requirements of the Ph.D. at the University of California, Los Angeles.

SUMMARY

1. *Dendraster excentricus* used the spines and tube feet to capture large food items such as algal fragments. In addition, the large bidentate pedicellariae were used to capture active prey.

2. Rejection of food occurred at the test surface or at the Y junction of the food grooves. The rejection response was well defined.

3. Specimens of *Dendraster* from a protected outer coast location ate primarily small crustaceans, diatoms, algal fragments, and sand grains. In a summer sample, diatoms were the most abundant item in the diet; in a winter sample, crustaceans predominated the diet.

4. Sand dollars from a protected outer coast sand dollar bed had more food of higher organic content in their guts than did sand dollars from two bay habitats.

5. Food passed through the stomach in 5 hr and through the entire gut in 2 days. Specimens of *Dendraster* from a protected outer coast habitat fed continuously.

6. Individuals of *Dendraster* were nonselective with respect to particle size in the range of 30 μm to 100 μm. Sixty per cent of the particles captured by specimens of *Dendraster* in the field were <180 μm in size.

LITERATURE CITED

Chia, F. S., 1969a. Some observations on the locomotion and feeding of the sand dollar *Dendraster excentricus* (Eschscholtz). *J. Exp. Mar. Biol. Ecol.*, **3**: 162–170.

Chia, F. S., 1969b. Histology of the pedicellariae of the sand dollar, *Dendraster excentricus* (Echinodermata). *J. Zool. (London)*, **157**: 503–507.

Goodbody, I., 1960. The feeding mechanism in the sand dollar, *Mellita sexiesperforata* (Leske). *Biol. Bull.*, **119**: 80–86.

Hyman, L. H., 1958. Notes on the biology of the five-lunuled sand dollar. *Biol. Bull.*, **114**: 54–56.

Ivlev, V. S., 1961. *Experimental ecology of the feeding of fishes.* Yale University Press, New Haven, Connecticut, 302 pp.

Lawrence, J. M., and L. Hughes-Games, 1972. The diurnal rhythm of feeding and passage of food through the gut of *Diadema setosum* (Echinodermata: Echinoidea). *Israel J. Zool.*, **21**: 13–16.

MacGinitie, G. E., and N. MacGinitie, 1949. *Natural history of marine animals.* McGraw-Hill, New York, 473 pp.

Merrill, R. J., and E. S. Hobson, 1970. Field observations of *Dendraster excentricus*, a sand dollar of Western North America. *Amer. Med. Natur.*, **83**: 595–624.

Reese, E. S., 1966. The complex behavior of echinoderms. Pages 157–217 in R. A. Boolootian, Ed., *Physiology of Echinodermata.* Wiley-Interscience, New York.

Reisman, A. W., 1965. The histology and anatomy of the intestinal tract of *Dendraster excentricus*, a clypeasteroid echinoid. *Master's thesis, University of California*, Los Angeles, California.

Sokal, R. R., and F. J. Rohlf, 1969. *Biometry.* W. H. Freeman and Co., San Francisco, 368 pp.

Sokolova, M. N., and A. P. Kusnetzov, 1960. On the feeding character and the role played by trophic factors in the distribution of the sea urchin *Echinarachnius parma. Zoologicheskii Zhurnal*, **39**: 1253–1256.

Timko, P. L., 1975. High density aggregation in *Dendraster excentricus* (Eschscholtz): Analysis of strategies and benefits concerning growth, age structure, feeding, hydrodynamics, and reproduction. *Doctoral dissertation, University of California*, Los Angeles, California, 323 pp. (*Diss. Abstr.*, **36(8)**: 3755B; Order no. 76–3059.)

Wagner, C. D., 1974. Recent and fossil echinoids of Alaska. *J. Paleontol.*, **48**: 105–123.

Reference: *Biol. Bull.,* **151**: 260–271. (August, 1976)

UTILIZATION OF ^{3}H-THYMIDINE TRIPHOSPHATE BY DEVELOPING STAGES OF *PECTINARIA GOULDII*

KENYON S. TWEEDELL

Department of Biology, University of Notre Dame, Notre Dame, Indiana 46556 and The Marine Biological Laboratory, Woods Hole, Massachusetts 02543

While earlier experiments (Tweedell, 1966) had demonstrated that ^{3}H-thymidine (^{3}H-TdR) was incorporated into nuclei of ovarian preoocytes in the polychaete *Pectinaria gouldii,* no uptake was observed in any oocyte stage during the vegetative period of oocyte growth and differentiation in the coelom. Nuclear DNA synthesis is apparently absent throughout the entire period of oogenesis until after fertilization. The failure of thymidine uptake during oocyte development might be attributed to the absence of thymidine kinase, other phosphorylating enzymes or the necessity of having a complete pool of DNA nucleosides. Utilization of ^{3}H-thymidine triphosphate (^{3}H-TTP) as an indicator of DNA synthesis seemed justified since uptake of deoxyribonucleoside triphosphates has been observed in membrane altered *E. coli* cells (Buttin and Kornberg, 1966; Moses and Richardson, 1970) and in growing cells of *Stentor coeruleus* (de Terra, 1967). Uptake of TTP *in vitro* by isolated nuclei of rat liver (Kaufman, Grisham and Stenstrom, 1972), rat thymus (Lagunoff, 1969) and HeLa cells (Friedman and Mueller, 1968) has also been obtained. Possible use of TTP by the developing embryo also seemed likely since isolated nuclei of embryonic, regenerating and neoplastic tissues showed a marked increase of TTP uptake above that found in normal rat nuclei (Lynch, Brown, Umeda, Langreth and Liebermann, 1970).

The potential incorporation of TTP into mitochondria of the oocyte of embryo was predicated by similar uptake *in vitro* by isolated mitochondria (Parsons and Simpson, 1967). To investigate each of these possibilities, developing stages of *Pectinaria* from the preoocyte stage through gastrulation were exposed to ^{3}H-TTP or ^{3}H-TdR alone or in combination with their respective complementary unlabeled nucleotides or nucleosides.

MATERIALS AND METHODS

Isotope presentation

Labeled nucleic acid precursors utilized were thymidine-methyl-H^3(TdR) (6.7 Ci/mM) in a concentration of 0.018 mg/ml and *d*-thymidine-methyl-H^3-5′ triphosphate (^{3}H-TTP) as a tetralithium salt (2.8 Ci/mM; Schwartz) or as a tetrasodium salt [15.7 Ci/mM (New England Nuclear)] at a concentration of 0.009 mg/ml. Experiments were repeated over a period of three summers with three separate lots of radioactive precursors.

In vivo *experiments*

Isotopes were diluted with sterile distilled water and injected through the cephalic placque into the coelomic cavity of adult animals after anesthesia with

50% ethanol (Tweedell, 1966). From 50 to 10 μCi of ^{3}H-TTP in 0.1 ml quantities were injected depending upon the volume of coelomic fluid. The average volume of coelomic fluid from large mature animals was determined to be 0.3 ml after cell removal. After returning the animals to their sand tests they were placed in running sea water for the pulse duration.

Nine adult females were injected with 5 to 10 μCi/animal of ^{3}H-TTP only and pulsed for periods of 0.5, 1, 2 and 4 hours. Several other adults received ^{3}H-TTP in the presence of the three unlabeled deoxynucleoside triphosphates for 0.5 to 2 hours, and one had an exposure of six days.

Postshedding

Oocytes from females were shed into sea water and germinal vesicle breakdown occurred in mature oocytes 15–20 min afterwards. The oocytes were washed and previously shed spermatozoa were added (6 drops/dish) 30 min after germinal vesicle breakdown. Fifteen minutes later the fertilized eggs were washed to remove excess spermatozoa and transferred into Millipore-filtered sea water. Aliquots of either oocytes, fertilized eggs or developmental stages were collected by light centrifugation and placed in 10 ml volumes of pasteurized sea water with 2.5 to 5 μCi/ml of either ^{3}H-TdR or ^{3}H-TTP. Either precursor was diluted with sterile distilled water and introduced as 0.1 ml quantities to give a final concentration of 0.0015 mM.

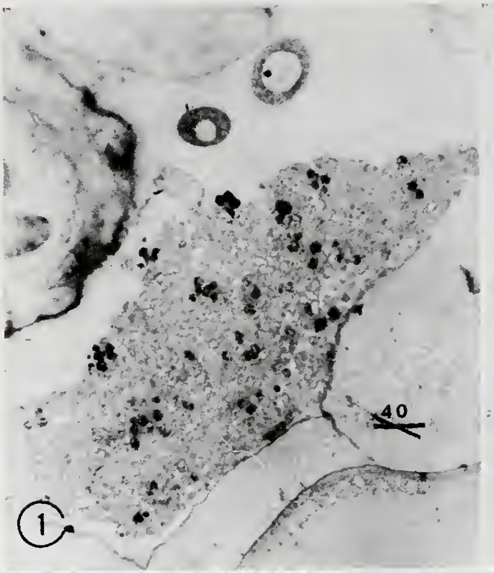
Unlabeled precursors

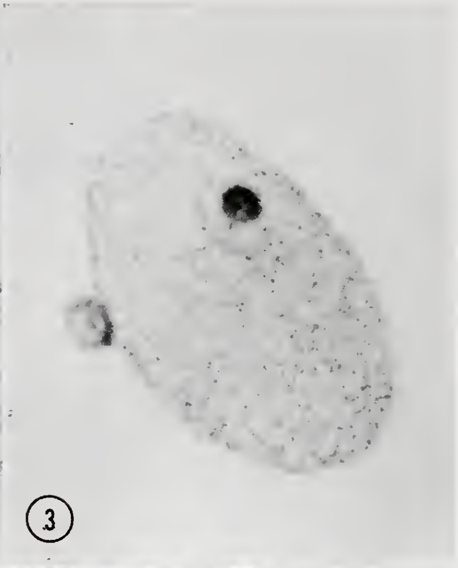
Samples of cells were also incubated with unlabeled nucleosides or nucleotides with the corresponding labeled nucleic acid precursor. Unlabeled *d*-adenosine, *d*-guanosine (0.01 mM) and *d*-cytosine (0.03 mM) were added with ^{3}H-thymidine to 10 ml of pasteurized sea water or up to 0.16 mM *in vivo*. In practice, they were contained in the sterile distilled water used to dilute the isotope. Unlabeled deoxynucleoside triphosphates, *d*-adenosine triphosphate (0.005 mM), *d*-guanosine triphosphate (0.005 mM) and *d*-cytosine triphosphate (0.005 mM) were added to ^{3}H-TTP in the incubation mixture.

Histology and autoradiography

Intact ovaries and oocytes after isotope exposure were preserved *in situ* with Kahle's fixative. The anterior one-third of the animal was embedded in methacrylate plastic and sectioned at 1 or 2 μ on a Sorvall J-B4 microtome. Eggs and embryos were also harvested after *in vivo* or external exposure to isotopes by swirling them into Kahle's fixative, followed by embedding in paraffin and sectioning at 5 μ. Selected sections of ovaries or eggs were treated with DNAase I (Sigma) prior to autoradiography in a concentration of 0.1 mg/ml in 0.05 M veronal acetate buffer, pH 6.8 with 0.003 M $MgCl_2$. Incubation was at 37° C for two hours.

Slides were dipped in Ilford L4 (1:1) or Kodak NTB2 emulsions. They were incubated at 4° C for two to three weeks, developed in D-72 for 3 min at 14° C, rinsed in water and fixed for 5 min. After washing they were stained in Jenner-Giemsa solution, differentiated in 0.1 N HCl and mounted in Permount.





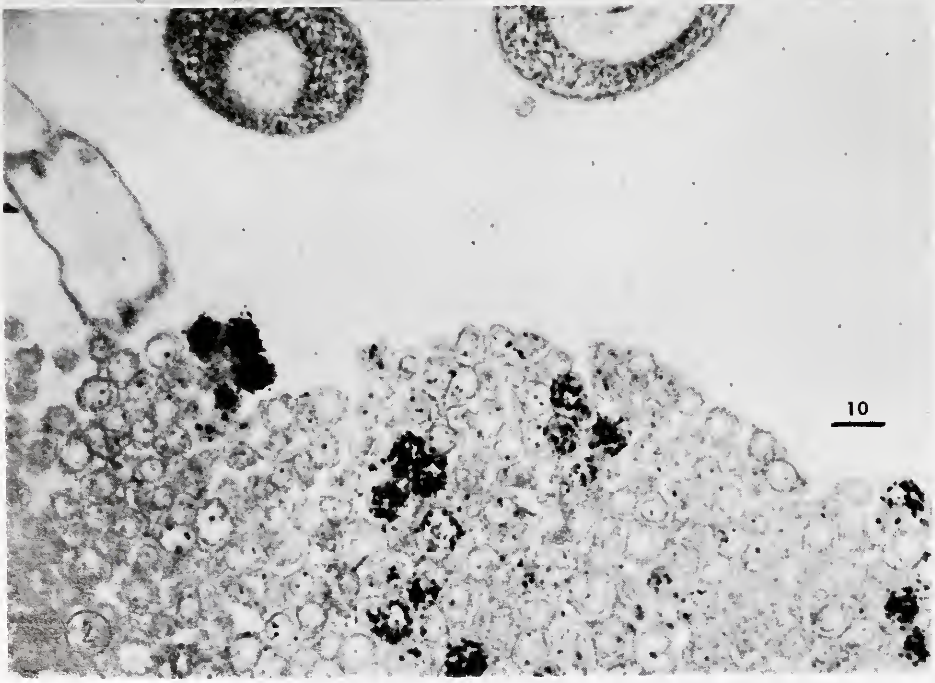


FIGURE 1. Scattered groups of preoocytes in the ovary after 1 hour exposure to ^{3}H-TTP; scale is 40 microns.

FIGURE 2. Distribution of ^{3}H-TTP uptake over nuclei of preoocytes of ovary. Some heavily labeled cells are seen budding from the edge of the ovary; scale is 10 microns.

FIGURE 3. Section through the trophozoite stage of a coelomic gregarine, *Urospora* sp. showing intense cytoplasmic labeling after exposure to ^{3}H-TTP for 1 hour. Note that the nucleus and an attached oocyte are unlabeled.

RESULTS

Since previous investigations of TTP incorporation *in vitro* had indicated the necessity of having all deoxyriboside triphosphates present, the concentration toxicity of either the unlabeled nucleotides or unlabeled nucleosides was determined on the development of *Pectinaria* eggs. Eggs were fertilized with preshed and washed sperm in 0.01 mM, 0.1 mM, and 1 mM concentrations of *d*-adenosine triphosphate (ATP), *d*-guanosine triphosphate (GTP) and *d*-cytidine triphosphate (CTP), prepared in pasteurized sea water prior to the introduction of the eggs. Equal volumes of fertilized eggs were added to 10 ml of each nucleotide dilution. After 22 hours all dilutions contained swimming gastrulae and larvae which indicated there was no toxic effect at these concentrations. Similar tests at the same dilutions were done on the four deoxynucleosides and they also failed to elicit a concentration toxicity.

Exposure to ^{3}H-TTP

The ovary. Normally, the ovary contains a mixed population of oogonial cells and preoocytes. In all cases after pulsing with ^{3}H-TTP a heavy nuclear label appeared over pairs or groups of cells scattered throughout the ovary; after four hours the label became very dense (Fig. 1). These cells, measuring 4–5 μ in diameter, have a very thin peripheral rim of cytoplasm, consequently the entire surface of the preoocytes sometimes appeared densely covered with silver grains. In thin 1 μ sections, when uptake was less dense, it was clearly located over the nucleus. Very heavily labeled cells were sometimes seen budding from the edge of the ovary into the coelom (Fig. 2), and this is the only example of free oocytes being labeled.

Both the distribution of silver grains and the amount of uptake appeared to be unaffected by the addition of complementary unlabeled nucleotides to ^{3}H-TTP. After treatment of the ovarian sections with DNAase I (0.1 mg/ml) for two hours prior to dipping, radioactivity disappeared from residual oocyte packets and host connective tissue. The nuclear label over the intact ovarian cells was quantitatively reduced but not completely eliminated.

Coelomic oocytes. Developmental stages of the primary oocyte labeled *in vivo* for 0.5, 1, 2 or 4 hours were examined both *in situ* within the coelomic cavity before germinal vesicle breakdown and after being induced to shed from an injected animal. The latter cells undergo germinal vesicle breakdown prematurely and progress into the first maturation division. Normally oocytes bud from the ovary into the coelomic fluid as small cell packets. After a period of cell growth within the packet, the cells separate and begin a phase of vegetative growth as separate, single oocytes until the mature oocytes are selectively taken into the nephromixia (Tweedell, 1966).

Sections taken through the coelom indicated that the full size range of oocyte packets remained unlabeled even after pulsing up to four hours. Single vegetative oocytes from the smallest individual oocytes to the largest mature primary oocyte also remained completely unlabeled. Application of either ^{3}H-TTP alone or with ATP, GTP and CTP gave the same results. After a six day exposure with 10 μCi of ^{3}H-TTP and the three unlabeled nucleotides, small, medium and fully mature oocytes were still unlabeled. Oocyte packets were also negative; however, some of the rosette clusters of coelomic cells and amoebocytes showed dense labeling over the entire cell surface. None of the oocyte packets showed any definitive label above the background.

One of the coinhabitants of the coelomic fluid with the developing oocytes is a prominent gregarine protozoan, *Urospora* sp. (Brasil, 1904). Simultaneous exposure of the adult vegetative form (trophozoite) *in vivo* to ^{3}H-dTTP revealed a heavy accumulation of silver grains over the entire cytoplasm (Fig. 3). The large, inactive nucleus was unaffected. None of the surrounding oocytes stages showed any indication of incorporation.

Postshedding exposure of oocytes to ^{3}H-TdR

Previous experiments (Tweedell, 1966) had demonstrated the lack of ^{3}H-TdR utilization by developing oocytes but active incorporation of ^{3}H-uridine; as a reaffirmation, new experiments were performed with ^{3}H-TdR and reinforced with unlabeled nucleosides. The incorporation of thymidine into cell fractions of regenerating liver was shown to be stimulated by a mixture of dAMP, dGMP and dCMP (Bollum, 1958). The presentation of ^{3}H-TdR and the unlabeled nucleosides *in vitro* to newly shed oocytes gave no evidence that the latter compounds implemented the uptake of ^{3}H-TdR by the oocytes.

Oocytes were also exposed to ^{3}H-TTP after being shed from females. Before germinal vesicle breakdown (12–15 min), they were placed in Millipore-filtered sea water with 2.5 to 4 μCi/ml of ^{3}H-TTP. Pulse times varied from 1.5 hours (20° C) to 4 hours at 4° C. No label appeared over the oocyte packets or over any of the solitary oocytes in vegetative growth. Mature oocytes were also refractory either before or after germinal vesicle breakdown. Again, the small coelomic rosette cells developed heavy nuclear labels.

To further detect any cytoplasmic localization of radioactivity the oocytes were allowed to undergo germinal vesicle breakdown before the introduction of ^{3}H-TTP. The cells were then placed into 2.5 to 4.0 μCi/ml of ^{3}H-TTP for 1 hour. After washing and chasing with cold thymidine, the cells were layered over a sucrose cushion and centrifuged at 28,700 × g at 0° C in order to stratify the cell components (Tweedell, 1962). While good granular stratification was obtained, there was no evidence of isotope uptake in any of the stratified layers.

Precursor uptake of ^{3}H-TdR by developing embryos

As an indication of the baseline activity of DNA synthesis during development, fertilized eggs were pulsed (0.5–1 hour) with ^{3}H-TdR (2.5 μCi/ml) after polar body formation following fertilization and during cleavage, blastula and gastrula stages. Other developing stages (4 cell to gastrula) were exposed to ^{3}H-thymidine with unlabeled *d*-nucleosides: *d*-adenosine, *d*-cytidine and *d*-guanosine. Stages were fixed immediately after pulsing. Heavy nuclear uptake of ^{3}H-TdR was very evident in all of the stages. Nuclei of swimming blastulae and randomly localized nuclei of gastrulae were heavily labeled. The intensity of nuclear uptake was greater in the latter stages at the same concentration and time of exposure. No difference was found in nuclear uptake when unlabeled *d*-nucleosides were used jointly with ^{3}H-Tdr.

^{3}H-TTP utilization

Fertilized eggs from four females were placed in 20 ml of filtered sea water containing ^{3}H-TTP (4 μCi/ml) from 20 min (2nd polar body formation) to 40 min (fusion of pronuclei) after fertilization. After pulsing for 45 min to 1 hour, the embryos were fixed immediately. Heavy nuclear label was found in all embryos of the 2, 4 and 8 cell stage (Fig. 4) after exposure to the labeled nucleotide. Unfertilized eggs (after germinal vesicle breakdown) and immature oocytes again failed to show any absorbance of the isotope.

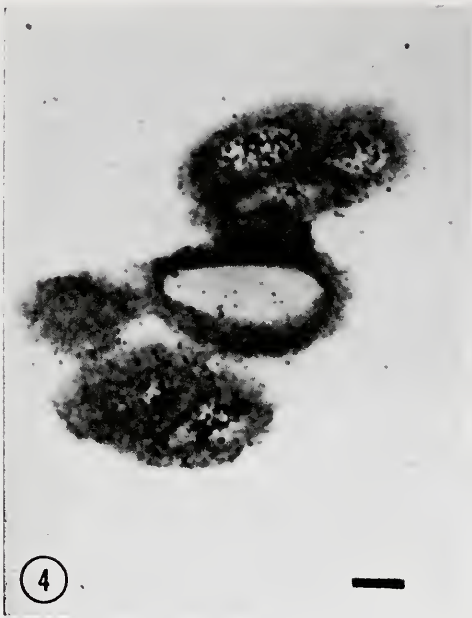
After fertilization and washing, other embryos in the 4, 8 and 16 cell stages were placed into ^{3}H-TTP (1.5 μCi/ml) with unlabeled *d*-nucleotides (dATP, dCTP and dGTP) for exposure times of 1, 2, 3 and 4.5 hours. Embryos pulsed for 1 hour and then fixed had reached the period of late cleavage; the nuclei of all stages were uniformly and intensely covered with silver grains (Fig. 5). Other embryos were chased with unlabeled dTTP after 1 hour and allowed to develop into early blastulae or swimming blastulae. The nuclei of all blastulae were moderately but evenly labeled after 2.5 hours (Fig. 6). The reduction in labeling probably resulted from dilution of the label by successive nuclear divisions. Free swimming ciliated blastulae were recovered after 4.5 hours and still showed a diluted distribution of label over nuclei. Again, the addition of the unlabeled *d*-nucleotides was not prerequisite, nor did they have any apparent effect on the amount of nuclear label.

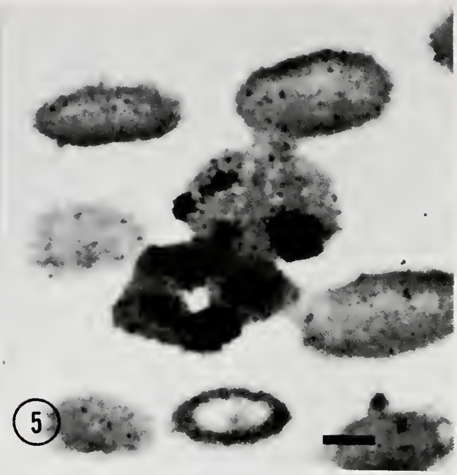
Fertilized eggs were also grown to the gastrulae stage (10 hours) and then placed in 2.5 μCi of ^{3}H-TTP plus *d*-nucleotides. After 1 hour they were harvested. Individual labeled nuclei again appeared but their distribution was nonuniform, appearing at random in sections generally toward the interior of the gastrulae (Fig. 7).

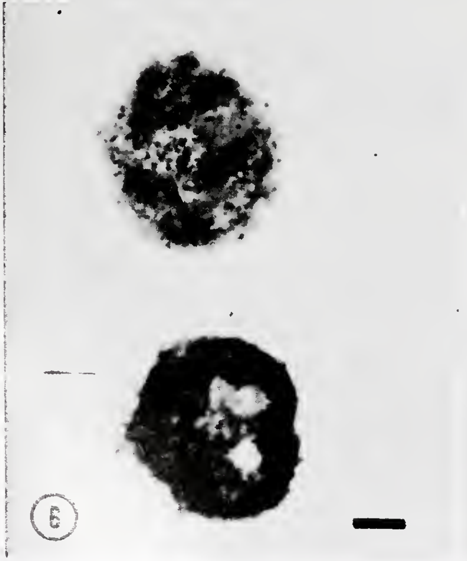
^{3}H-TTP and thymidine

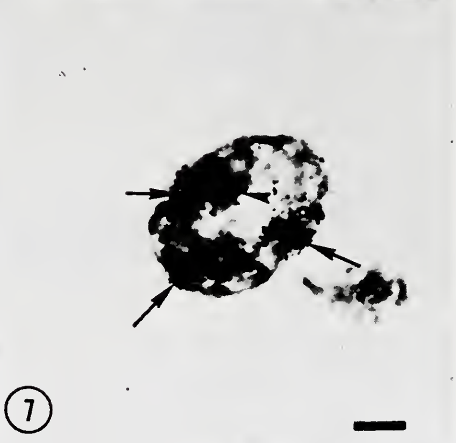
The possibility that ^{3}H-TTP was being converted to ^{3}H-TdR before incorporation into the cell was investigated by adding an excess of unlabeled TdR to the labeled ^{3}H-TTP. If conversion to ^{3}H-TdR did occur, the labeled nucleoside would have to compete with the cold thymidine pool and thus the final nuclear label should be greatly reduced. Free swimming blastulae (5 hours old) were collected by centrifugation, washed in sterile sea water and added to 25 μCi of ^{3}H-TTP (0.0015 mmole) in 10 ml of pasteurized sea water. Unlabeled thymidine (0.83 mmole) was added simultaneously. As an additional control, another dish received ^{3}H-TTP plus 0.7 mmole of thymidine, *d*-adenosine, *d*-guanosine and *c*-cytidine. After 1.5 hours, the blastulae were recovered, washed and fixed. The addition of unlabeled thymidine with or without the complete nucleosides had no visible effect on the amount of nuclear uptake. Scattered nuclei still gave strong evidence of ^{3}H-TTP utilization with no apparent reduction in the nuclear label (Fig. 8).

As a measure of the effectiveness of the thymidine dilution effect, parallel experiments were conducted by direct exposure of the ovaries *in situ* to 2.5 μCi/ml of ^{3}H-Tdr (specific activity: 20 Ci/mM) at a concentration of 0.00025 mmole. In a second series the labeled nucleoside was combined with undiluted thymidine at a concentration of 0.125 mmole. Exposure lasted for 0.5 hours.


4


5


6


7

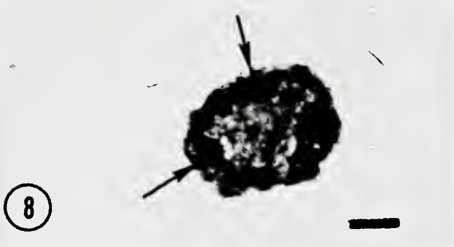

8

FIGURE 4. Nuclear incorporation of ^{3}H-TTP into 2 and 4 cell embryos after 40 min exposure. Mature oocyte nucleus (center) shows no uptake; scale is 12 microns.

FIGURE 5. Embryos pulsed with ^{3}H-TTP for 1 hour and fixed in late cleavage-early blastula stages, showing heavy nuclear uptake. Mature oocyte is without label; scale is 15 microns.

FIGURE 6. Light label over nuclei of swimming blastulae 2.5 hours after 1 hour pulse chase with ^{3}H-TTP in the 4 cell stage; scale is 14 microns.

FIGURE 7. A gastrula pulsed for 1 hour in ^{3}H-TTP plus *d* nucleotides. Scattered nuclei (arrows) show incorporation; scale is 14 microns.

Label over the ovarian cells with ^{3}H-TdR alone was scattered but the intensity was greatly diminished in the cells exposed to an excess of unlabeled thymidine.

DISCUSSION

The lack of isotope incorporation from ^{3}H-TTP into the nuclei of all coelomic stages of the developing oocytes of *Pectinaria* parallels the earlier finding with ^{3}H-TdR (Tweedell, 1966). Even the addition of all deoxynucleosides of ^{3}H-TdR in the present experiments failed to show thymidine uptake in the oocytes. The results suggest that the oocytes are impermeable to nucleosides and nucleotides or that DNA synthesis is absent or deficient. Yet, earlier experiments showed that ^{3}H-uridine penetrated the oocyte quite readily. Gurdon and Woodland (1968) did find a lack of DNA synthesis after injection of primer DNA and ^{3}H-TdR into growing or full sized oocytes of *Xenopus*. They concluded that DNA polymerase is absent or inactive in the egg cytoplasm at the time of germinal vesicle breakdown. It is also possible that the required kinases are not available during oocyte formation in *Pectinaria*, but oocyte impermeability to TTP is still a possibility.

Simultaneous *in vivo* exposure of ^{3}H-TTP produced cytoplasmic uptake in the parasitic gregarine protozoan *Urospora* found among the developing oocytes in the coelom of the adult *Pectinaria*. It may be significant that the same trophozoit stage pulsed with ^{3}H-TdR failed to show either cytoplasmic or nuclear label. Similar cytoplasmic labeling of *Stentor coeruleus* with ^{3}H-TTP during all stages of the cell growth cycle was observed by de Terra (1967). In the current experiments the cytoplasmic labeling by ^{3}H-TPP may be direct incorporation by mitochondria. Isolated rat liver mitochondria will incorporate ^{14}C-dTTP into DNA (Parsons and Simpson, 1967; Kalf, D'Agostimo and Hunter, 1971) identified as closed circle DNA (Parsons, Karol and Simpson, 1968). Uptake of ^{3}H-TTP has also been reported in isolated mitochondria of *Neurospora* (Koke, Malhotra and Bryan, 1970). However, incorporation into the protozoan *Urospora* conceivably could result from symbiotic or ingested microorganisms.

The obvious lack of cytoplasmic uptake of either ^{3}H-TdR or ^{3}H-TTP into the growing oocytes of *Pectinaria* implies that biosynthsis of mitochondrial DNA has also been completed. Oocytes typically possess many mitochondria. Mature oocytes of the amphibian egg contain 10^5 times as many mitochondria as somatic cells (Chase and Dawid, 1972). The amount of DNA in the unfertilized sea urchin egg is estimated as seven times the haploid amount of which 80–90% is mitochondrial DNA (Piko, Tyler and Vinograd, 1967). Isolated mitochondria from unfertilized mature eggs of the Loach showed little uptake of ^{3}H-TTP but induction of breaks in the mitochondrial DNA with an antibiotic, bruneomycin, could stimulate incorporation (Gause and Mikhailov, 1973).

Even though the growing oocytes in the vegetative phase showed no indication of taking up ^{3}H-TTP, the nuclear uptake in the preoocytes of the ovary repeated that found earlier with ^{3}H-TdR (Tweedell, 1966). Exposure of the ovary to ^{3}H-TTP also resulted in intense labeling over random parts of clumps of pre-

FIGURE 8. A free swimming blastula exposed to ^{3}H-TTP plus an excess of unlabeled thymidine for 1.5 hours. Several nuclei (arrows) were still heavily labeled with no apparent reduction in label: scale is 18 microns.

oocytes. These cells, labeled in the S-period prior to oocyte maturation in the G_2 interval, were resistant to DNAase treatment. This characteristic of nonextractibility is indicative of replictaing DNA (Friedman and Mueller, 1968).

Fertilized eggs and developing embryos of *Pectinaria* (2 cell through the gastrulae) all showed vigorous nuclear labeling after pulsing with ^{3}H-TTP that was indistinguishable from that obtained with ^{3}H-TdR. Cells pulsed at the 2 cell stage could be traced, with minimal label dilution, into the gastrula stage. Direct utilization of thymidine triphosphate into intact nuclei of living cells *in vivo* has been reported rarely, although nuclear uptake of ^{3}H-dTTP was indicated in 18 hr growing *Stentor* cells (de Terra, 1967). Likewise, changing the culture conditions to make cells "leaky" (Buttin and Kornberg, 1966) or more permeable to nucleotides (Moses and Richardson, 1970) has permitted uptake of TTP into cells of *E. coli.*

A similar correlation might then be advanced that *in vivo* use of TTP by embryonic cells is somewhat unique. Thus far, the reported observations have been limited to free-living single cell systems (*E. coli* and *Stentor*). The experiments on developing embryos of *Pectinaria* suggest that the labeled nucleotide, ^{3}H-TTP is incorporated into the nuclear DNA of all stages from fertilization through the gastrula stage.

There are numerous examples of the direct use of TTP by isolated nuclei. Under appropriate *in vitro* conditions isolated nuclei of either HeLa cells (Friedman and Mueller, 1968), rat thymus (Lagunoff, 1969), and rat liver (Lynch *et al.*, 1970; Cook, 1972) are able to incorporate thymidine triphosphate. Similarly, the herterochromatic fractions from mouse liver nuclei incorporate TTP into DNA (Klose and Flickinger, 1972).

The *in vitro* studies do suggest there is a strong difference in the degree of TTP utilization between normal nuclei and those obtained from fetal, regenerating or neoplastic tissues. Lynch *et al.* (1970) reported that isolated liver nuclei from regenerating liver incorporate ^{3}H-TTP at 10 times the rate of normal liver nuclei. However, Kaufman *et al.* (1972) found that while both normal and regenerating liver nuclei were active *in vitro,* normal nuclei are inactive *in vivo,* the latter reflecting unscheduled DNA synthesis.

When DNA synthesis of isolated liver nuclei is compared with strains of Morris hepatoma nuclei, the latter utilized ^{3}H-TTP from six to ten times more *in vitro* and exceeded parallel incorporation of ^{3}H-TdR *in vivo* (Ove, Coetzee and Morris, 1971). The uptake of ^{3}H-dTTP into DNA of nuclei isolated from Ehrlich ascites tumor cells was found to increase in proportion to the amount of X-irradiation of cells *in vivo* (Matsudaira and Furundo, 1971). Likewise, the antitumor antibiotic phleomycin will also stimulate incorporation of TTP into nuclei isolated from HeLa cells, osteosarcoma cells and transformed cells (Friedman, Stern and Rose, 1974). It is relevant that isolated rat liver mitochondria which incorporate dTTP or dATP *in vitro* (Kalf *et al.*, 1971) are also stimulated by cytoplasmic factors present in the postmicrosomal fraction of rat and mouse tumors and in regenerating and fetal rat liver, but they are absent from normal adult liver.

Entrance of the nucleotide macromolecules into intact developing cells is an unlikely problem. There is good evidence that DNA can penetrate mammalian or other cells in tissue culture (Ledoux, 1965; Robins and Taylor, 1968; Hill and

Hillova, 1971). Exogenous high molecular weight DNA (HMW ^{3}H-DNA) can even enter cleaving mouse embryos *in vitro* more proficiently than ^{3}H-TdR (Snow and McLaren, 1974).

Another difference and one of the basic requirements reported in most of the previous investigations for uptake of TTP into leaky cells or subcellular fractions has been the dependence on the presence of all four deoxyribonucleotides for utilization of labeled dTTP. The addition of the 3 nucleoside triphosphates seemed to have little effect on labeling of nuclei in early embryos of *Pectinaria* after exposure to ^{3}H-TTP.

The direct utilization of ^{3}H-TTP might be interpreted as a result of the dephosphorylation of ^{3}H-TTP to ^{3}H-TdR with its subsequent incorporation into DNA of the nucleus. This would either presume the release of phosphatases from the cell or dephosphorylation of TTP after cell entry. The application of cold thymidine 550 times in excess of ^{3}H-dTTP was an attempt to show that TTP was not being dephosphorylated before uptake. If TTP was being reduced to thymidine before uptake, the cold thymidine should successfully compete with the radioactive thymidine and reduce the label but in fact there was no decrease. The lack of any apparent reduction in the nuclear label from ^{3}H-TTP mitigates against the conversion of TTP to TdR by exogenous phosphatases. If such conversion was taking place the unlabeled thymidine pool would be expected to dilute out the labeled thymidine so that less nuclear labeling occurred.

On the other hand, if there was not a conversion of ^{3}H-TTP to ^{3}H-TdR, then this implies that ^{3}H-TTP either has a competitive advantage over the unlabeled TdR or that it was inhibiting TdR. Such a possibility exists since the presence of TTP has a feedback inhibition on the thymidine kinase in mammalian cells (Breitman, 1965; Kit, Dubbs and Frearson, 1964) and in *Xenopus* ooctyes (Woodland, 1969). The effect seems to be specific for dTTP (Ives, Morse and Potter, 1963) as opposed to other nucleotides.

SUMMARY

1. Exposure of developing *Pectinaria* embryos to ^{3}H-TTP results in immediate nuclear label over all postfertilization stages.

2. Intense nuclear label also occurs over preoocytes or oogonia within the ovary after *in vivo* ^{3}H-TTP pulsing.

3. No nuclear uptake is obtained in either the packet or solitary vegetative oocyte with either ^{3}H-TTP or ^{3}H-TdR until after fertilization. There was no detection of mitochondrial DNA synthesis in oocytes but cytoplasmic labeling of a gregarine protozoan occurred *in vivo* with ^{3}H-TTP.

4. The addition of complementary nucleosides or nucleotides has no effect on the quantitative uptake of either radioactive precursor into oocytes or developing embryos.

5. These experiments suggest that ^{3}H-TTP is being utilized by dividing cells of *Pectinaria* embryos. Actual incorporation of ^{3}H-TTP rather than degradation of TTP to TdR was implied from observations showing that no reduction of nuclear ^{3}H-TTP occurred in the presence of excess unlabeled thymidine.

LITERATURE CITED

BOLLUM, F. J., 1958. Incorporation of thymidine triphosphate into deoxyribonucleic acid by a purified mammalian enzyme. *J. Amer. Chem. Soc.*, **80**: 1966.

BRASIL, L., 1904. Contribution a la connaissance de l'appareil digestif des Annelides Polychaetes. L'epithélium intestinal de la *Pectinaire*. *Arch. Zool. Exp. Gen. Ser. 4,* **2**: 91–255.

BREITMAN, T. R., 1963. The feedback inhibition of thymidine kinase. *Biochim. Biophys. Acta.*, **67**: 153–155.

BUTTIN, G., AND A. KORNBERG, 1966. Enzymatic synthesis of deoxyribonucleic acid. XXI. Utilization of deoxyribonucleoside triphosphates by *Escherichia coli* cells. *J. Biol. Chem.*, **241**: 5419–5427.

COOK, R. T., 1972. Nucleoside triphosphate inhibition of DNA synthesis in isolated rat hepatic nuclei. *Exp. Cell Res.*, **73**: 533–536.

CHASE, J. W., AND I. B. DAWID, 1972. Biogenesis of mitochondria during *Xenopus laevis* development. *Develop. Biol.*, **27**: 504–518.

DE TERRA, N., 1967. Macronuclear DNA synthesis in *Stentor:* regulation by a cytoplasmic initiator. *Proc. Nat. Acad. Sci. U.S.A.*, **57**: 607–614.

FRIEDMAN, D. L., AND G. C. MUELLER, 1968. A nuclear system for DNA replication from synchronized HeLa cells. *Biochim. Biophys. Acta.*, **161**: 455–468.

FRIEDMAN, R. M., R. STERN, AND J. A. ROSE, 1974. Phleomycin stimulation of thymidine triphosphate incorporation by animal cell nuclei. *J. Nat. Cancer Instit.*, **52**: 693–697.

GAUSE, G. G., AND V. S. MIKHAILOV, 1973. State of the DNA synthesizing system in isolated mitochondria from mature eggs of the loach (*Misgurnus fossilis*). *Biochim. Biophys. Acta.*, **324**: 189–198.

GURDON, J. B., AND H. R. WOODLAND, 1968. The cytoplasmic control of nuclear activity in animal development. *Biol. Rev.*, **43**: 233–267.

HILL, M., AND J. HILLOVA, 1971. Recombinational events between exogenous mouse DNA and newly synthesized DNA strands of chicken cells in culture. *Nature* (*New Biology*), **231**: 261–265.

IVES, D. A., P. A. MORSE, AND V. R. POTTER, 1963. Feedback inhibition of thymidine kinase by thymidine triphosphate. *J. Biol. Chem.*, **238**: 1467–1474.

KALF, B. F., M. A. D'AGOSTINO, AND G. R. HUNTER, 1971. DNA biosynthesis by isolated mitochondria stimulation by cytoplasmic factors from neoplastic and regenerating tissues. *Cancer Res.*, **31**: 2054–2058.

KAUFMAN, D. G., J. W. GRISHAM, AND M. L. STENSTROM, 1972. Unscheduled incorporation of ^{3}H-TTP into DNA of isolated rat liver nuclei. *Biochim. Biophys. Acta.*, **272**: 212–219.

KIT, S., D. R. DUBBS, AND P. M. FREARSON, 1964. Decline of thymidine kinase activity in stationary phase mouse fibroblast cells. *J. Biol. Chem.*, **240**: 2565–2573.

KLOSE, J., AND R. A. FLICKINGER, 1972. Incorporation of deoxythymidine triphosphate into DNA of fractions of mouse liver *in vitro*. *Exp. Cell Res.*, **73**: 236–239.

KOKE, J. R., S. K. MALHOTRA, AND L. E. BRYAN, 1970. Factors influencing incorporation of ^{3}H-TTP into an acid precipitable product by isolated mitochondria of *Neurospora crassa*. *Cytobios*, **2**: 19–27.

LAGUNOFF, D., 1969. Thymidine-5-triphosphate incorporation into DNA by rat thymus nuclei. *Exp. Cell Res.*, **55**: 53–56.

LEDOUX, L., 1965. Uptake of DNA by living cells. *Progr. Nucl. Acid Res. Mol. Biol.*, **4**: 231–267.

LYNCH, W. E., R. F. BROWN, T. UMEDA, S. G. LANGRETH, AND I. LIEBERMANN, 1970. Synthesis of deoxyribonucleic acid by isolated liver nuclei. *J. Biol. Chem.*, **245**: 3911–3916.

MATSUDAIRA, H., AND I. FURUNDO, 1971. Increase in the incorporation of ^{3}H thymidine triphosphate into the DNA of nuclei isolated from Ehrlich ascites-tumour cells irradiated *in vivo*. *Int. J. Radiat. Biol.*, **19**: 393–397.

MOSES, R. E., AND C. C. RICHARDSON, 1970. Replication and repair of DNA in cells of *Escherichia coli* treated with toluene. *Proc. Nat. Acad. Sci. U. S. A.*, **67**: 674–681.

OVE, P., M. L. COETZEE, AND H. P. MORRIS, 1971. DNA synthesis and the effect of sucrose in nuclei of host liver and Morris hepatomas. *Cancer Res.*, **31**: 1389–1395.

Parsons, P., and M. V. Simpson, 1967. Biosynthesis of DNA by isolated mitochondria: incorporation of thymidine triphosphate-2-C^{14}. *Science,* **155**: 91–93.

Parsons, P., M. Karol, and M. V. Simpson, 1968. DNA biosynthesis in mitochondria: DNA replication by isolated rat liver mitochondria. *J. Cell Biol.,* **39**: 102a–103a.

Piko, L., A. Tyler, and J. Vinograd, 1967. Amount, location, priming capacity, circularity and other properties of cytoplasmic DNA in sea urchin eggs. *Biol. Bull.,* **132**: 68–90.

Robins, A. B., and D. M. Taylor, 1968. Nuclear uptake of exogenous DNA by mammalian cells in culture. *Nature,* **217**: 1226–1231.

Snow, M. H. L., and A. McLaren, 1974. The effect of exogenous DNA upon cleaving mouse embryos. *Exp. Cell Res.,* **86**: 1–8.

Tweedell, K. S., 1962. Cytological studies during germinal vesicle breakdown of *Pectinaria gouldii* with vital dyes, centrifugation and fluorescence microscopy. *Biol. Bull.,* **123**: 424–449.

Tweedell, K. S., 1966. Oocyte development and incorporation of H^3 thymidine and H^3 uridine in *Pectinaria (Cistenides) gouldii. Biol. Bull.,* **131**: 516–538.

Woodland, H. R., 1969. The phosphorylation of thymidine by oocytes and eggs of *Xenopus laevis* Daudin. *Biochim. Biophys. Acta.,* **186**: 1–12.

Continued from Cover Two

4. ***Literature Cited.*** The list of references should be headed LITERATURE CITED, should conform in punctuation and arrangement to the style of recent issues of THE BIOLOGICAL BULLETIN, and must be typed *double-spaced* on separate pages. Note that citations should include complete titles and inclusive pagination. Journal abbreviations should normally follow those of the U. S. A. Standards Institute (USASI), as adopted by BIOLOGICAL ABSTRACTS and CHEMICAL ABSTRACTS, with the minor differences set out below. The most generally useful list of biological journal titles is that published each year by BIOLOGICAL ABSTRACTS of those abstracted (most recent issue: November, 1972). Foreign authors, and others who are accustomed to use THE WORLD LIST OF SCIENTIFIC PERIODICALS, may find a booklet published by the Biological Council of the U.K. (obtainable from the Institute of Biology, 41 Queen's Gate, London, S.W.7, England, U.K. at £0.65 or $1.75) useful, since it sets out the WORLD LIST abbreviations for most biological journals with notes of the USASI abbreviations where these differ. CHEMICAL ABSTRACTS publishes quarterly supplements of additional abbreviations. The following points of reference style for THE BIOLOGICAL BULLETIN differ from USASI (or modified WORLD LIST) usage:

A. Journal abbreviations, and book titles, all underlined (for *italics*)

B. All components of abbreviations with initial capitals (not as European usage in WORLD LIST *e.g. J. Cell. Comp. Physiol.* NOT *J. cell. comp. Physiol.*)

C. *All* abbreviated components must be followed by a period, whole word components ***must not*** (not strictly as USASI usage, *i.e. J. Cancer Res.*)

D. Space between all components (*e.g. J. Cell. Comp. Physiol.* not *J.Cell.Comp.Physiol.*)

E. We strongly recommend that more unusual words in journal titles be spelled out in full, rather than employing lengthy, peculiar "abbreviations" or new abbreviations invented by the author. For example, use *Rit Vísindafjélags Islendinga* without abbreviation. Even in more familiar languages, *Z. Vererbungslehre* is preferred to *Z. VererbLehre* (WORLD LIST) or *Z. Vererbungsl.* (USASI). *Accurate and complete communication of the reference is more important than minor savings in printing costs.*

F. All single word journal titles in full (*e.g. Veliger, Ecology, Brain*).

G. The order of abbreviated components should be the same as the word order of the complete title (*i.e. Proc.* and *Trans.* placed where they appear, not transposed as in some BIOLOGICAL ABSTRACTS listings).

H. Spell out *London, Tokyo, Paris, Edinburgh, Lisbon, etc.* where part of journal title.

I. Series letters *etc.* immediately before volume number.

J. A few well-known international journals in their preferred forms rather than WORLD LIST or USASI usage (*e.g. Nature, Science, Evolution* NOT *Nature, Lond.; Science, N.Y.; Evolution, Lancaster, Pa.*)

K. The correct abbreviation for THE BIOLOGICAL BULLETIN is *Biol. Bull.*

5. ***Figures.*** The dimensions of the printed page, 5 by 7⅜ inches, should be kept in mind in preparing figures for publication. Illustrations should be large enough so that all details will be clear after appropriate reduction. Explanatory matter should be included separately in legends as far as possible, although the axes should always be numbered and identified on the illustration itself. Figures should be prepared for reproduction as either line-cuts or halftones; no other methods will be used. Figures to be reproduced as line-cuts should be drawn in black ink on white paper, good quality tracing cloth or plastic, or blue-lined coordinate paper; those to be reproduced as halftones should be mounted on board, and both designating numbers or letters and scale-bars should be affixed directly on the figures. We recommend that halftones submitted to us be mounted prints made at about 1½ times the linear dimensions of the final printing desired (the actual best reductions are achieved from copy in the range from 1¼ to 2 times the linear dimensions). As regards line-blocks, originals can be designed for even greater reductions but are best in the range 1½ to 3 times. *All* figures should be numbered in consecutive order, with no distinction between text and plate-figures. The author's name should appear on the reverse side of all figures, and the inked originals for line-blocks must be submitted for block-making.

6. ***Mailing.*** Manuscripts should be packed flat. All illustrations larger than 8½ by 11 inches must be accompanied by photographic reproductions or tracings that may be folded to page size.

Reprints. Reprints may be obtained at cost; approximate prices will be furnished by the Managing Editor upon request.

CONTENTS

Annual Report of the Marine Biological Laboratory 1

Cooley, Lynn, Dana R. Crawford and Stephen H. Bishop
Urease from the lugworm, *Arenicola cristata* 96

Elvin, David W.
Seasonal growth and reproduction of an intertidal sponge, *Haliclona permollis* (Bowerbank) 108

Forward, Richard B., Jr.
A shadow response in a larval crustacean 126

Gwilliam, G. F.
The mechanism of the shadow reflex in Cirripedia. III. Rhythmical patterned activity in central neurons and its modulation by shadows 141

Kahler, George A., Frank M. Fisher, Jr., and Ronald L. Sass
The chemical composition and mechanical properties of the hinge ligament in bivalve molluscs 161

Mackie, George O., C. L. Singla, and Catherine Thiriot-Quiévreux
Nervous control of ciliary activity in gastropod larvae 182

McLaren, Ian A.
Inheritance of demographic and production parameters in the marine copepod *Eurytemora herdmani* 200

Mills, Claudia E.
Podocoryne selena, a new species of hydroid from the Gulf of Mexico, and a comparison with *Hydractinia echinata* 214

Pardy, R. L.
The production of aposymbiotic hydra by the photodestruction of green hydra zoochlorellae 225

Sweeney, Beatrice M.
Circadian rhythms in corals, particularly Fungiidae 236

Timko, Patricia L.
Sand dollars as suspension feeders: a new description of feeding in *Dendraster excentricus* 247

Tweedell, Kenyon S.
Utilization of ^{3}H-thymidine triphosphate by developing stages of *Pectinaria gouldii* 260

Volume 151 Number 2

THE
BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

Woods Hole, Mass.

Editorial Board

JOHN B. BUCK, National Institutes of Health
JOHN O. CORLISS, University of Maryland
DONALD P. COSTELLO, Woods Hole, Massachusetts
JOHN D. COSTLOW, Duke University
PHILIP B. DUNHAM, Syracuse University
CATHERINE HENLEY, National Institutes of Health
GEORGE O. MACKIE, University of Victoria
HOWARD A. SCHNEIDERMAN, University of California, Irvine
RALPH I. SMITH, University of California, Berkeley
GROVER C. STEPHENS, University of California, Irvine
CARROLL M. WILLIAMS, Harvard University
EDWARD O. WILSON, Harvard University

W. D. RUSSELL-HUNTER, Syracuse University
Managing Editor

OCTOBER, 1976

Printed and Issued by
LANCASTER PRESS, Inc.
PRINCE & LEMON STS.
LANCASTER, PA.

THE BIOLOGICAL BULLETIN

THE BIOLOGICAL BULLETIN is issued six times a year at the Lancaster Press, Inc., Prince and Lemon Streets, Lancaster, Pennsylvania.

Subscriptions and similar matter should be addressed to THE BIOLOGICAL BULLETIN, Marine Biological Laboratory, Woods Hole, Massachusetts. Agent for Great Britain: Wheldon and Wesley, Limited, 2, 3 and 4 Arthur Street, New Oxford Street, London, W. C. 2. Single numbers, $7.00. Subscription per volume (three issues), $18.00, (this is $36.00 per year for six issues).

Communications relative to manuscripts should be sent to Dr. W. D. Russell-Hunter, Marine Biological Laboratory, Woods Hole, Massachusetts 02543 between May 23 and September 1, and to Dr. W. D. Russell-Hunter, P.O. Box 103, University Station, Syracuse, New York 13210, during the remainder of the year.

Copyright © 1976, by the Marine Biological Laboratory
Second-class postage paid at Lancaster, Pa.

INSTRUCTIONS TO AUTHORS

THE BIOLOGICAL BULLETIN accepts original research reports of intermediate length on a variety of subjects of biological interest. In general, these papers are either of particular interest to workers at the Marine Biological Laboratory, or of outstanding general significance to a large number of biologists throughout the world. Normally, review papers (except those written at the specific invitation of the Editorial Board), very short papers (less than five printed pages), preliminary notes, and papers which describe only a new technique or method without presenting substantial quantities of data resulting from the use of the new method cannot be accepted for publication. A paper will usually appear within four months of the date of its acceptance.

The Editorial Board requests that manuscripts conform to the requirements set below; those manuscripts which do not conform will be returned to authors for correction before review by the Board.

1. ***Manuscripts.*** Manuscripts must be typed in double spacing (*including* figure legends, foot-notes, bibliography, etc.) on one side of 16- or 20-lb. bond paper, 8½ by 11 inches. They should be carefully proof-read before being submitted and all typographical errors corrected legibly in black ink. Pages should be numbered. A left-hand margin of at least 1½ inches should be allowed.

2. ***Tables, Foot-Notes, Figure Legends, etc.*** Tables should be typed on separate sheets and placed in correct sequence in the text. Because of the high cost of setting such material in type, authors are earnestly requested to limit tabular material as much as possible. Similarly, foot-notes to tables should be avoided wherever possible. If they are essential, they should be indicated by asterisks, daggers, etc., rather than by numbers. Foot-notes are not normally permitted in the body of the text. Such material should be incorporated into the text where appropriate. Explanations of figures should be typed double-spaced and placed on separate sheets at the end of the paper.

3. ***A condensed title*** or running head of no more than 35 letters and spaces should be included.

Continued on Cover Three

NOTICE TO SUBSCRIBERS

Effective with the first issue of Volume 152 (February, 1977), the subscription price for THE BIOLOGICAL BULLETIN will be raised. Subscription per volume (three issues) will be $22.00 (this is $44.00 per year for six issues); single numbers will be $8.00 each. Continuing 1977 subscriptions for which payment has actually been received before November 1, 1976 will be honored at the old rate.

Vol. 151, No.2 October 1976

THE
BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

ANTENNULAR CHEMOSENSITIVITY IN THE SPINY LOBSTER, *PANULIRUS ARGUS:* COMPARATIVE TESTS OF HIGH AND LOW MOLECULAR WEIGHT STIMULANTS

Reference: *Biol. Bull.,* **151**: 273–282. (October, 1976)

B. W. ACHE, Z. M. FUZESSERY, AND W. E. S. CARR

Department of Biological Sciences, Florida Atlantic University, Boca Raton, Florida 33431,
and Whitney Marine Laboratory, University of Florida, Rt. 1, Box 121,
St. Augustine, Florida 32084

The role of proteins and other macromolecules as feeding stimuli for marine invertebrates is not clearly defined. Macromolecules can be effectively deployed in the aquatic environment (Wilson, 1970), but the extent to which marine organisms utilize such molecules is the subject of some controversy among students of chemical communication (Heeb, 1973). Behavioral studies implicating macromolecules acting as feeding stimulants in polychaetes (Magnum and Cox, 1971), natantians (Carr and Gurin, 1975), asteroids (Heeb, 1973), and gastropods (Gurin and Carr, 1971; Carr, Hall, and Gurin, 1974) counter the trend of thinking established by earlier studies that low molecular weight, readily diffusable organic molecules are the predominate feeding stimuli for marine invertebrates (for review see Lenhoff and Lindstedt, 1974). Further investigation of the stimulatory role of macromolecules is in order.

Electrophysiological analysis of chemoreceptor sensitivity to macromolecular stimuli provides a novel approach to evaluating the potential of macromolecules as feeding stimuli. Case (1964) demonstrated that polyglutamic acid and two proteins were not stimulatory to dactyl chemoreceptors of the brachyuran, *Cancer antennarius.* From Wilson's (1970) consideration, proteins serving as contact chemical stimuli raises fewer conceptual problems than proteins serving as distance stimuli where increased emission rates, lowered receptor thresholds, and/or current-facilitated dispersal are necessary to offset the low diffusion coefficients of proteins in water. An analysis of distance chemoreceptors would therefore be particularly appropriate to understanding the potential stimulatory capacity of macromolecules.

Behavioral and electrophysiological data implicate the antennules of decapod crustaceans as distance chemoreceptors (Hazlett, 1971). The spiny lobster, *Panulirus argus,* with its elongated antennular filaments is well suited for physiological analysis of antennular chemoreception. Previous electrophysiological studies indicate antennular sensitivity to organic substances of low molecular weight in this species (Laverack, 1964; Levandowsky and Hodgson, 1965), and in the

American lobster, *Homarus americanus* (Ache, 1972; Shepheard, 1974). These substances at least partially elicit feeding behavior in lobsters of both species (personal observation; Mackie, 1973; McLeese, 1973). Behavioral studies of Carr and Gurin (1975) indicate that the chemical nature of major feeding stimulus for the shrimp, *Palaemonetes pugio,* varied with the type of stimulatory extract employed. The latter necessitates a survey of a series of stimulus types to fully understand potential macromolecular sensitivity of antennular chemoreceptors.

The present study surveys the sensitivity of antennular chemoreceptors of *Panulirus argus* to extracts and body fluids of potential food organisms both before and after fractionation by ultrafiltration. Data presented indicate that receptors sensitive to these extracts occur on the lateral antennular filaments and that, for all extracts and fluids tested, components of less than *ca.* 10,000 molecular weight are significantly more stimulatory than the components of higher molecular weight. Further, the stimulatory levels of the lower molecular weight components do not differ significantly from those of the full extracts or fluids.

Materials and Methods

Animal maintenance

Locally caught specimens of *Panulirus argus* were maintained in 150 gal. tanks of recirculating artificial sea water and fed frozen shrimp every third day.

Recording procedure

The preparation was the aesthetasc-containing distal 5–6 cm section excised from the lateral antennular filaments of adult lobsters. The filament was clamped through a silicon septum in a lucite recording chamber with the aesthetasc-containing annuli projecting into a tubular compartment carrying a continuous flow (10 ml/min) of reagent-grade artificial sea water into which various potential stimulants were introduced. The septum also isolated the stimulus-containing flow from a second compartment containing about 10 ml saline into which the filament's proximal end projected. Oxygen-saturated *Panulirus* saline (Mulloney and Selverston, 1974) perfused the filament *via* intralumenal cannulation. Perfusion extended viability of the otherwise short-lived preparation up to 3 hr post excision. Removal of the 3–4 most proximal cuticular annuli exposed the antennular nerve for subsequent teasing of fine nerve bundles onto platinum monopolar hook-type recording electrodes. Lifting into air provided sufficient resistance for recording 30–70 μV potentials from active chemosensory fibers. A Ag-AgCl pellet grounded the 10 ml bath. Neural activity was displayed on conventional recording equipment and stored on magnetic tape for subsequent photography and analysis.

Preparation and ultrafiltration of stimulants

The following live marine animals were used for preparing the indicated extracts or body fluids:

Mollusca	
Coquina *(Donax variabilis)*	entire crushed animals
Oyster *(Crassostrea virginica)*	soft parts mantle fluid

Arthropoda	
Blue crab (*Callinectes sapidus*)	claw muscle hemolymph serum
Pink shrimp (*Penaeus dourarum*)	abdominal muscle
Echinodermata	
Sea urchin (*Arbacia punctulata*)	soft parts
Chordata	
Striped mullet (*Mugil cephalus*)	muscle

Extracts (3 : 1, volume : weight) were prepared by cutting up tissue in cold 1% NaCl and shaking for 30 minutes in an ice bath followed by centrifugation at 4° C and filtration of supernatants through Whatman #1 paper. A 100 : 1 extract of Tetramin (Tetra Sales Corp., Hayward, California-dry flaked fish food containing both animal and plant material) was prepared by adding flakes to 1% NaCl and treating as above. Mantle fluid of oyster and hemolymph serum of crab were prepared as described by Carr *et al.* (1974).

Each extract or body fluid was ultrafiltered at 4° C with 30 psi of N_2 through an Amicon UM-10 membrane retaining molecules greater than *ca.* 10,000 molecular weight. Each ultrafiltration provided two fractions as follows: a retentate containing substances of greater than *ca.* 10,000 mol wt and an ultrafiltrate containing smaller molecules. Each of these fractions and remainder of each total extract or fluid was divided into 5 ml aliquots and frozen until used. Single aliquots were thawed just prior to use and serially diluted with reagent-grade artificial sea water to provide a concentration series of that stimulant type. All stimuli were brought to ambient temperature (23° C), the temperature of the carrier flow, immediately before use since preliminary studies indicated a marked temperature sensitivity of antennular chemoreceptors.

Experimental protocol

Brief applications of a particular extract or fluid (and a seawater control) identified active chemosensory units as bundle searching proceeded. Bundles containing active units were then further subdivided until discrete unit activity could be resolved over background noise. An effective, yet nonsaturating concentration of test stimulus for a particular bundle was selected and 50 μl volumes each of the total extract, the retentate fraction, the filtrate fraction, and a repeated total extract were then applied to the preparation at the test concentration. A 2 min wash period separated each 50 μl application. Failure to obtain a terminal response to full extract equal in intensity to the initial full extract response voided the data of a series. Individual antennular preparations were tested with a single extract. The data presented on a given type of extract or fluid represent units from at least five different antennular preparations. To test for the possible loss of components labile to the ultrafiltration process, selected antennular preparations were used to compare the activity of each total extract or fluid with that of a 1 : 1 mixture of the retentate and ultrafiltrate.

TABLE I

Comparison of antennular chemosensitivity in P. argus *to extracts or fluids of potential food organisms before and after ultrafiltration into* >ca. *10,000 (retentate) and <ca. 10,000 (filtrate) molecular weight fractions.*

Extract type	Number of tests	$\bar{X}$ activity ratios		F*	*t*-test scores	
		Retentate: total	Filtrate: total		Retentate *vs* filtrate*	Filtrate *vs* total extract**
Coquina	8	0.37	0.98	14.43	6.62	0.21
Oyster-Fluid	9	0.28	0.95	12.81	5.70	0.40
Oyster-Tissue	14	0.22	0.84	11.10	6.45	1.28
Crab-Muscle	9	0.37	0.86	11.27	6.43	1.42
Crab-Serum	9	0.28	1.04	8.74	5.00	0.29
Shrimp	11	0.20	0.97	17.53	7.39	0.34
Urchin	8	0.12	0.96	17.09	58.56	0.34
Mullet	10	0.29	0.92	18.09	7.76	0.86
Tetramin***	7	0.15	1.00	11.21	4.17	0.00

* All values significant at the 0.01 level (single-sided).

** All values not significant, with $P \geqq 0.10$ level (single-sided).

*** A commercially-prepared fish food, containing both animal and plant material.

Data analysis

Lacking evidence as to what parameter(s) of afferent spike trains encode(s) chemosensory information in the lobster, we used the total spike count as the least variable and, tentatively, the most descriptive measure of responsiveness. The "response" of a nerve bundle therefore represents the total number of spikes elicited in that bundle on application of a given stimulus. Variability in the response of nerve bundles to a particular extract and to its ultrafiltration fractions was determined with a single classification analysis of variance and, if significant, specific treatments were compared further by a *t*-test. Analyses were performed on raw data. Tabulated values represent "mean activity ratios" in which the responses of a bundle to filtrate and to retentate are normalized to that bundle's mean extract response (the average response to initial and terminal total extract applications) prior to averaging.

RESULTS

Data are reported from 85 nerve bundles, each containing from one to approximately 12 active chemosensory units. Table I summarizes the overall results. For each of the nine types of extracts and fluids, the mean response to filtrate fractions was significantly greater than to retentate fractions. Comparisons of the response magnitudes for filtrates and retentates *vs* total extracts or fluids show ratios ranging from 0.84 to 1.04 for filtrates and only 0.12 to 0.37 for retentates. Further, for each type of extract or fluid, the mean response to filtrate fractions did not differ significantly from that elicited by the total extract. Thus, while both high and low molecular weight components are stimulatory, the major stimulants are present in the lower molecular weight component.

The greater stimulatory capacity of the lower molecular weight fractions did not result from the ultrafiltration procedure itself. In control trials, combined retentate and filtrate fractions elicited responses not significantly different from those elicited by the total extract or fluid. In 3 to 7 selected nerve bundles tested for each type of extract or fluid, 1 : 1 mixtures of the two fractions elicited responses with magnitudes 0.83 to 1.28 times that of the respective total extract responses.

Analysis of single unit activity provides further insight into the nature of the observed responses. Twenty-four single units were selected from the data presented in Table I and further analyzed. These include responses of bundles containing only single active chemoreceptors, and single active chemoreceptors selected from multi-fiber bundles containing sufficiently few units to allow discrete unit resolution (see Figure 1). Chemosensitive units were consistently small diameter fibers judging from their 100 μV-range spike amplitudes recorded with hook electrodes. Table II summarizes the single unit responses. The data were first analyzed to describe the basic response of actual chemoreceptors to total extract stimulation. Individual units responded consistently although interunit variability was appreciable. In general, units increased their rates of firing on total extract stimulation from spontaneous levels of 0–2 impulses/sec to maximum levels of 8–82 impulses/sec, subsequently decaying back to pre-stimulation levels over 0.7–12 seconds (Fig. 1, top 2 traces). Response duration and maximum spike frequency increased with stimulus concentration from thresholds of 10^{-4} to 10^{-5} times stock

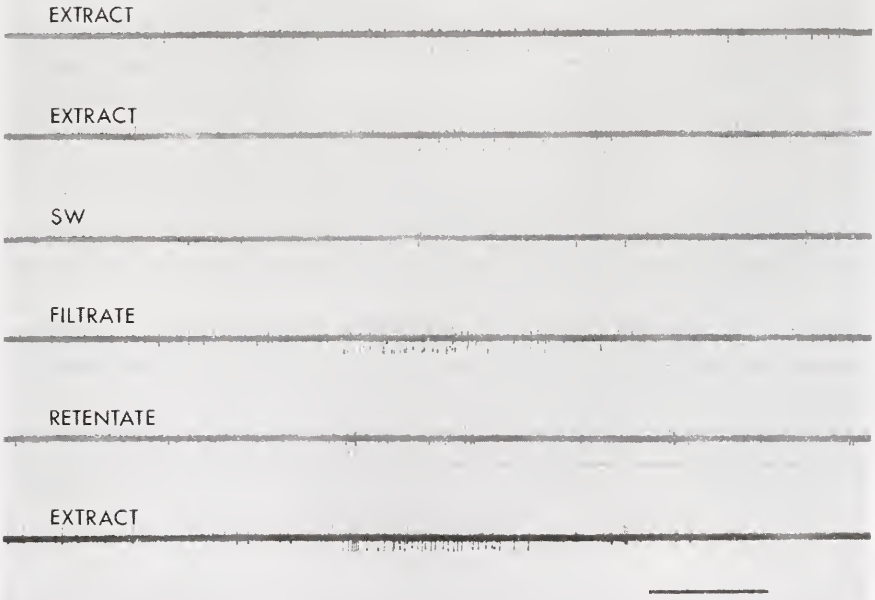


FIGURE 1. Extracellular records of chemosensory activity in *P. argus* lateral antennular filament in response to Tetramin (see text for composition) extract stimulation, 10^{-2} times stock concentration. Time calibration is 1.0 sec.

TABLE II

Single unit responses of antennular chemoreceptors in P. argus *to extracts or fluids of potential food organisms before and after ultrafiltration into* >ca. *10,000 (retentate) and* <ca. *10,000 (filtrate) molecular weight fractions.*

Unit number	Extract type	Number of spikes			Activity ratios	
		$\bar{X}$ total	Retentate	Filtrate	Filtrate: $\bar{X}$ total	Retentate: $\bar{X}$ total
1	Coquina	42	10	44	1.05	0.24
2	Oyster-tissue	108	7	79	0.73	0.06
3	Oyster tissue	15	6	12	0.80	0.40
4	Oyster tissue	20	7	14	0.70	0.40
5	Oyster tissue	9	3	3	0.33	0.33
6	Oyster tissue	33	0	30	0.91	0.00
7	Oyster-fluid	13	1	12	0.92	0.08
8	Oyster-fluid	34	14	50	1.47	0.41
9	Oyster fluid	68	0	29	0.43	0.00
10	Crab-muscle	9	14	8	0.89	1.56
11	Crab-muscle	38	9	23	0.61	0.24
12	Crab-serum	9	2	3	0.33	0.22
13	Crab-serum	116	6	99	0.85	0.05
14	Shrimp	170	14	144	0.84	0.08
15	Shrimp	16	5	17	1.06	0.31
16	Shrimp	122	8	82	0.67	0.07
17	Shrimp	84	6	92	1.10	0.07
18	Urchin	28	11	29	1.04	0.39
19	Urchin	16	1	18	1.13	0.06
20	Mullet	23	0	20	0.87	0.00
21	Mullet	26	25	24	0.92	0.96
22	Tetramin	28	0	28	1.00	0.00
23	Tetramin	33	3	30	0.91	0.11
24	Tetramin	35	1	32	0.91	0.03

extract concentrations. Response latencies obtained in our apparatus varied from 2.0 to 12.4 sec post-stimulus introduction. (The minimum delay for the stimulus front to traverse the entire length of the excised filament was 1 sec as determined visually by dye flow.).

Single units were then analyzed for differences in sensitivity to the two fractions. Twenty units responded more to filtrate than to retentate stimulation, one responded more to retentate stimulation, and three showed essentially no difference in response. Filtrate: total activity ratios among units more sensitive to filtrate stimulation varied from 0.43–1.47. No evidence of temporal patterning differing from that described for the total extracts was apparent in either the filtrate or the retentate responses.

DISCUSSION

Comparisons of the stimulatory capacity of high and low molecular weight fractions from 9 extracts and body fluids show clearly that in each case the major stimulants for antennular chemoreceptors of *Panulirus argus* are substances of less than *ca.* 10,000 mol wt. If, as considered below, the antennules are primarily distance chemoreceptors, it seems unlikely that macromolecules play a major role in the attraction of *P. argus* to distant food sources. Unfortunately, behavioral correlates of the present study on *P. argus* are not available. Behavioral studies of food-finding in two other decapods, *Homarus gammarus* and *Carcinus maenas,* demonstrate that synthetic mixtures of low mol wt components present in food organisms provide attractants as effective as aqueous extracts of the organisms (Mackie, 1973; Shelton and Mackie, 1971). In both of these studes, however, macromolecular components were not systematically eliminated as potential attractants. Carr and Gurin (1975) concluded that the attraction of the natantian, *Palaemonetes pugio,* to human serum was due to the combined effects of both macromolecules (proteins) and substances of less than 1000 mol wt. A mixture of the 37 major low mol wt components of human serum was only about one-eighth as effective as the total serum itself. Further analysis of chemosensitivity in *Palaemonetes* using extracts similar to those employed in the present study showed that whereas the major stimulants in three extract types were components of less than 1000 mol wt, those in two other extract types were components of greater than 1000 mol wt. Our current data with *P. argus* support the hypothesis that the chemical signals used for food-finding in this species are not macromolecules, though they may be small proteins or peptides.

Bardach (1975) recently reviewed and listed the known and suspected chemical signals of marine organisms. Molecules of less than 1000 mol wt dominate the list. As noted by Carr *et al.* (1974) most studies, both behavioral and physiological, focus specifically on such small molecules as potential stimulants without systematically eliminating components of higher mol wt by "working down" from a complete stimulant such as an aqueous extract or a body fluid. Several investigators have fractionated aqueous extracts or body fluids that stimulate feeding behavior and have shown that, in certain cases, macromolecules make important contributions to their activity. Gurin and Carr (1971) studied the chemical stimulation of feeding behavior in the gastropod, *Nassarius obsoletus,* and found that the major stimulant in oyster mantle fluid was a glycoprotein of *ca.* 100,000 mol wt that was effective at concentrations as low as 10^{-10} M. Magnum and Cox (1971) reported a glycoprotein of about 20,000 mol wt that contributed significantly to the feeding response of the tube-dwelling polychaete, *Diopatra cuprea.* Heeb (1973) found protein fractions of > 100,000 mol wt from two mollusc extracts to be major stimulants of the "humping reflex" and food-searching behavior in the seastar, *Asterias forbesii.* Alarm responses in the aquatic gastropod, *Helisoma duryi,* are apparently elicited by conspecific tissue components of *ca.* 100,000 mol wt (Snyder, 1967, cited in Bardach, 1975). Since at least some of these macromolecular stimuli elicit oriented locomotion or exhibit extremely low effective concentrations (10^{-10} M in *Nassarius*), the difference between the findings cited above and those of the present study cannot be explained on the basis of distance chemoreception *vs* contact or close-range chemoreception. Regarding prior demonstrations of the stimulatory capacity of macromolecules, Mackie (1975) proposed the interesting idea that the slow fade out time of large molecules, resulting from their low diffusion constants, may make them more favorable as chemical signals in slow-moving organisms such as gastropods and seastars than in faster-moving organisms such as decapod crustaceans.

It is generally assumed that crustacean antennules function in distance chemoreception, *i.e.,* in the detection of low concentrations of water-borne odorants which alert and/or initiate oriented behavior in the recipient organism (for review see Hazlett, 1971). Exceptions apparently exist, as antennules are reported not to mediate food location in a freshwater crayfish (Ameyaw-Akumfi and Hazlett, 1975). That lateral antennular filaments effect distance chemoreception in *P. argus* is supported by ablation studies (unpublished data) in which lobsters lacking this appendage fail to locate a source of shrimp extract in an olfactometer designed specifically to assay the chemotaxic components of feeding behavior. Similar results are reported for the American lobster (McLeese, 1973). As noted elsewhere (Ache, 1975), ablation with subsequent loss of response is not a definitive technique as the presumed chemoreceptor could be mediating nonchemical signals required concomitantly with chemosensory input *via* other receptor structures for orientation. This alternative has not been ruled out in *P. argus* but knowledge that chemoreceptors with thresholds as low as 10^{-11} M glycine and L-glutamic acid occur on the lateral filament of *P. argus* (Price and Ache, in preparation), it remains a less probable explanation of the ablation results.

Previous attempts at recording chemosensory activity from crustacean antennules have been limited by preparation viability. Our results, obtained from perfused preparations, extend and support those of previous studies that antennular (lateral filament) chemoreceptors of *P. argus* (Laverack, 1964; Levandowsky and Hodgson, 1965), the American lobster (Ache, 1972, Shepheard, 1974) and the brachyuran, *Plagusia dentipes,* (Ai and Takei, 1973) are small diameter fibers yielding fast, 30–100 μV extracellular spikes. Small spikes coupled with long response latencies and concentration-dependent decay periods characterize chemosensory responses from those of the ever-present mechanosensory fibers that occur in the same fiber bundles.

No attempt was made to compare response spectra of the individual fibers to the various extract types, but unit responses can be compared relative to the retentate and ultrafiltrate fractions used in the present study. While all but one of the single units responded much more strongly to the filtrate fractions, most of the units also showed some weak response to the retentate fractions. It is likely that part of the weak response obtained with retentates was due to the presence of small concentrations of low molecular weight substances not removed by the ultrafiltration process. Another explanation for the activity of the retentates is that proteins and other large molecules in these fractions possess functional groups which bind with receptor sites for low mol wt compounds. However, the overall contribution of the large molecules to the effectiveness of the total extracts and fluids was minimal as indicated by the fact that each ultrafiltrate, devoid of molecules of greater than *ca.* 10,000 mol wt, was virtually as stimulatory as the unfractionated extract or fluid. Further analyses are in progress concerning the specific nature of the substances in shrimp ultrafiltrate that stimulate antennular chemoreceptors in *P. argus* (Johnson and Ache, in preparation). These analyses indicate that the major stimulants have molecular weights of less than 1000 and are amino acid-like in nature.

The authors would like to thank Mr. Bruce Johnson for his help in initiating this project.

SUMMARY

Antennular chemoreceptors in the spiny lobster, *P. argus,* were surveyed electrophysiologically for responsiveness to natural stimuli of different molecular weights to gain further insight into the stimulatory role of macromolecules. Extracts and body fluids from eight potential food organisms were prepared and tested both before and after fractionation by ultrafiltration. Data presented verify chemosensitivity of lateral antennular filaments and show that for all extract types. the components of low molecular weight (< *ca.* 10,000) were significantly more stimulatory than the components of higher molecular weight; and stimulus values of low molecular weight fractions did not differ significantly from those of the unfractionated extracts.

LITERATURE CITED

ACHE, B., 1972. Amino acid receptors in the antennules of *Homarus americanus. Comp. Biochem. Physiol.,* **42A**: 807–811.

ACHE, B., 1975. Antennular mediated host location by symbiotic crustaceans. *Mar. Behav. Physiol.,* **3**: 125–130.

AI, N., AND M. TAKEI, 1973. Chemoreception in the antennual organ of the marine crab. *Zool. Mag.,* **82**: 59–63.

AMEYAW-AKUMFI, C., AND B. HAZLETT, 1975. Sex recognition in the crayfish, *Procambarus clarkii. Science,* **190**: 1225–1226.

BARDACH, J., 1975. Chemoreception of aquatic animals. Pages 121–132 in D. Denton and J. Coghlan, Eds., *Olfaction and taste, V.* Academic Press, New York.

CARR, W., E. HALL, AND S. GURIN, 1974. Chemoreception and the role of proteins: A comparative study. *Comp. Biochem. Physiol.,* **47A**: 559–566.

CARR, W., AND S. GURIN, 1975. Chemoreception in the shrimp, *Palaemonetes pugio:* comparative study of stimulating substances in human serum. *Biol. Bull.,* **148**: 380–392.

CASE, J., 1964. Properties of the dactyl chemoreceptors of *Cancer antennarius* Stimpson and *C. prouctus* Randall. *Biol. Bull.,* **127**: 428–446.

GURIN, S., AND W. CARR, 1971. Chemoreception in *Nassarius obsoletus:* the role of specific stimulatory proteins. *Science,* **174**: 293–295.

HAZLETT, B., 1971. Antennule chemosensitivity in marine decapod crustacea. *J. Anim. Morphol. Physiol.,* **18**: 1–10.

HEEB, M., 1973. Large molecules and chemical control of feeding behavior in the starfish, *Asterias forbesi. Helgolaender Wiss. Meeresunters,* **24**: 425–435.

LAVERACK, M., 1964. The antennular sense organs of *Panulirus argus. Comp. Biochem. Physiol.,* **13**: 301–321.

LENHOFF, H., AND K. LINDSTEDT, 1974. Chemoreception in aquatic invertebrates. Pages 143–175 in P. Grant and A. Mackie, Eds., *Chemoreception in marine organisms.* Academic Press, New York.

LEVANDOWSKY, M., AND E. HODGSON, 1965. Amino acid and amine receptors of lobsters. *Comp. Biochem. Physiol.,* **16**: 159–161.

MACKIE, A., 1973. The chemical basis of food detection in the lobster, *Homarus gammarus. Mar. Biol.,* **21**: 103–108.

MACKIE, A., 1975. Chemoreception. Pages 69–105 in D. Malins and J. Sargent, Eds., *Biochemical and biophysical perspectives in marine biology, Vol. 2.* Academic Press, New York.

MAGNUM, C., AND C. COX, 1971. Analysis of the feeding response in the onuphid polychaete, *Diopatra cuprea. Biol. Bull.,* **140**: 215–229.

MCLEESE, D., 1973. Olfactory responses of lobsters (*Homarus americanus*) to solutions from prey species and to seawater extracts and chemical fractions of fish muscle and effects of antennule ablation. *Mar. Behav. Physiol.,* **2**: 237–249.

MULLONEY, B., AND A. SELVERSTON, 1974. The organization of the stomatogastric ganglion of the spiny lobster. I. Neurons driving the lateral teeth. *J. Comp. Physiol.*, **91**: 1–32.

SHELTON, R., AND A. MACKIE, 1971. Studies on the chemical preferences of the shore crab, *Carcinus maenas*. *J. Exp. Mar. Biol. Ecol.*, **7**: 41–49.

SHEPHEARD, P., 1974. Chemoreception in the antennules of the lobster, *Homarus americanus*. *Mar. Behav. Physiol.*, **2**: 261–273.

WILSON, E. O., 1970. Chemical communication within animal species. Pages 133–135 in E. Sondheimer and J. Simeone, Eds., *Chemical ecology*. Academic Press, New York.

Reference: *Biol. Bull.,* **151** : 283–296. (October, 1976)

ULTRASTRUCTURE OF THE ATYPIC MUSCLES ASSOCIATED WITH TERMINALIAL INVERSION IN MALE *AEDES AEGYPTI* (L)[1]

BORIS I. CHEVONE AND A. GLENN RICHARDS

Department of Entomology, Fisheries and Wildlife, University of Minnesota,
St. Paul, Minnesota 55108

Rotation of the male genitalia occurs commonly among the Diptera (reviews by Crampton, 1941; Griffiths, 1972). Two primary types of rotation have been described, a permanent 360° circumversion (Cyclorrapha) and a 180° inversion (most Nematocera and most Brachycera). Among the Nematocera, the 180° inversion may be temporary, occurring only during copulation, or permanent, occurring shortly after adult emergence. Rotation frequently occurs in the intersegmental membrane between abdominal segments VII and VIII, but may involve more than one intersegment of the posterior part of the abdomen.

General reviews of the biology of the Culicidae (Marshall, 1938; Matheson, 1941) suggest that a permanent 180° rotation of the genitalia occurs in all species of this family. Christophers (1915) described inversion of the hypopygium in *Culex fatigans* and later (1922) unsuccessfully attempted to demonstrate abdominal muscles capable of causing rotation. Hodapp (1960) also failed to find muscles suitably positioned to cause rotation in *Aedes aegypti,* although he did experimentally determine that the mechanism for partial rotation was located in the rotating segments themselves.

Fittkau (1971) and Dordel (1973) have recently investigated genitalia rotation in several species of Chironomidae. In contrast to the culicids, both a temporary and a permanent 180° inversion are found among the chironomids. Fittkau and Dordel described complex muscle systems as the rotational mechanism in the posterior part of the abdomen of male chironomids.

The present report is part of an investigation of the rotational process in male *Aedes aegypti.* Ultrastructural changes occurring in the abdominal intersegmental membrane cuticle during rotation are presented in a separate communication (Chevone and Richards, in preparation). This report describes two pairs of opposed muscles found in the rotating region of male *A. aegypti.* These muscles have not been previously reported. The position of these muscles and the histological changes they undergo during rotation indicate that they probably provide the rotational force for inversion of the terminalia.

MATERIAL AND METHODS

A stock of *A. aegypti* (Rutgers strain) was maintained in an environmentally controlled room at 28 ± 1.5° C; 70 ± 10% relative humidity; and a 12 hour photophase. Eggs were vacuum-hatched for 30 minutes (Barbosa and Peters, 1969), and larvae developed in distilled water (250 larvae/liter) fed on dead, dried

---

[1] Paper no. 8972, Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul, Minnesota 55108, U. S. A. Acknowledgment is made for financial support from the National Institutes of Health (Grant AI-09559).

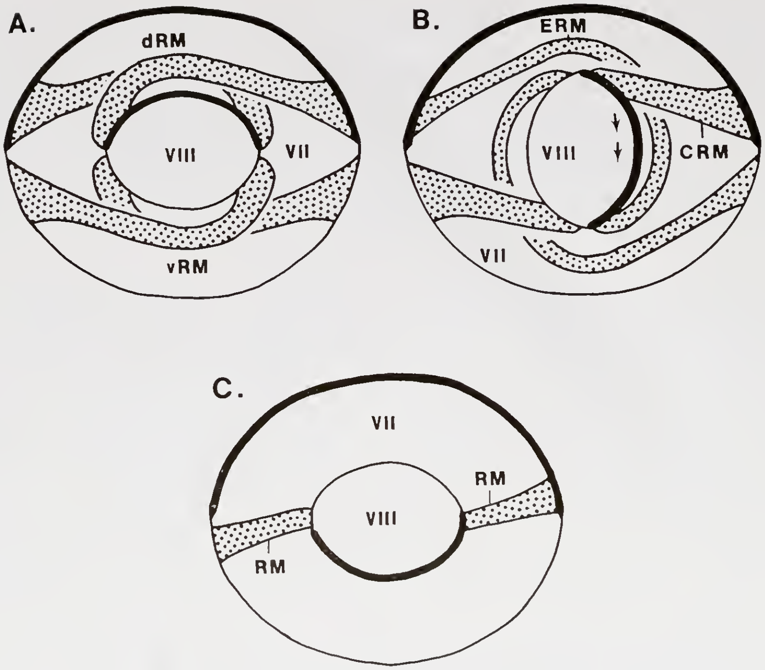


FIGURE 1A-C. Schematic representation of the paired muscles associated with rotation during stages of terminalia inversion. CRM represents contracting rotational muscles; dRM, dorsal rotational muscles; ERM, stretched rotational muscles; RM, muscles producing rotation; vRM, ventral rotational muscles; VII, abdominal segment VII; VIII, abdominal segment VIII.

FIGURE 1A. Cross section of the abdomen through the rotating region in a 32 to 33-hour pharate adult male (48 hours after pupation) showing the pre-rotational position of the paired muscles.

FIGURE 1B. Cross section of the abdomen through the rotating region at 90° of rotation. One member of each muscle set is partially contracted (CRM) and the other has become stretched (ERM). Arrows indicate the direction of rotation.

FIGURE 1C. Cross section of the abdomen through the rotating region 24 hours after the completion of rotation. The stretched muscles are no longer present. The contracted rotational muscles (RM) are positioned to prevent further movement of abdominal segment VIII.

Brewer's yeast. Larval development required 5 to 6 days and pupal development 52 to 75 hours.

For light microscopy, the posterior part of abdomens of males of a known age or degree of rotation were fixed in Dietrich's (Kahle's) fluid. Specimens were dehydrated *via* a graded *n*-butanol-ethanol series (Lee, 1950), embedded in paraffin (mp 61° C) containing 5% beeswax and 5% bayberry wax, and sectioned at 5 or 6 μm. Sections were stained with either Mallory's triple stain or Heidenhain's iron hematoxylin.

For electron microscopy, males of a known age or degree of rotation were first chilled at 10° C for five minutes. Portions of the abdomen (2 to 3 segments each) were fixed at 0 to 4° C for one to two hours in aqueous 2.5% glutaraldehyde buffered with 0.06 M phosphate buffer to pH 7.4. Sucrose was added to the fixation to give a final calculated osmolarity of 520 mOsmol. Specimens were then washed for one hour in sucrose-phosphate buffer, post-fixed for four to twelve hours in 1% OsO_4 buffered with 0.06 M phosphate buffer, dehydrated *via* an ethanol-propylene oxide series, and embedded in Epon 812. Sections were cut on a Reichert ultramicrotome at 80 to 100 nm, stained with lead citrate and uranyl acetate, and examined in a Philips 300 transmission electron microscope at 60 kV.

RESULTS

General aspects of rotation

Rotation of the male terminalia was observed at 28° C and found to correspond with Hodapp's (1960) report. Rotation begins 1 to 4 hours after adult emergence and reaches 90° in 6 to 12 hours after emergence. A variable resting period of 1 to 6 hours occurs at the 90° position and rotation is completed in 18 to 24 hours. Rotation may be either clockwise or counterclockwise in a 1:1 ratio (66:62, respectively, in specimens tabulated in this study).

Light microscopy of atypic muscles associated with terminalia rotation

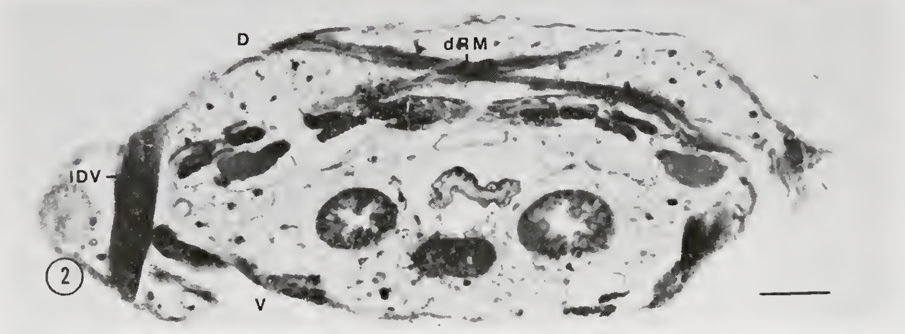
In cross sections of pharate adults (42 to 48 hours after pupation) or newly emerged male imagoes, two sets of opposed, crossed muscles, one set dorsal and one set ventral, are present in the rotating intersegmental region (Figs. 1A and 2). Similar muscles were not found in the few female specimens that were examined.

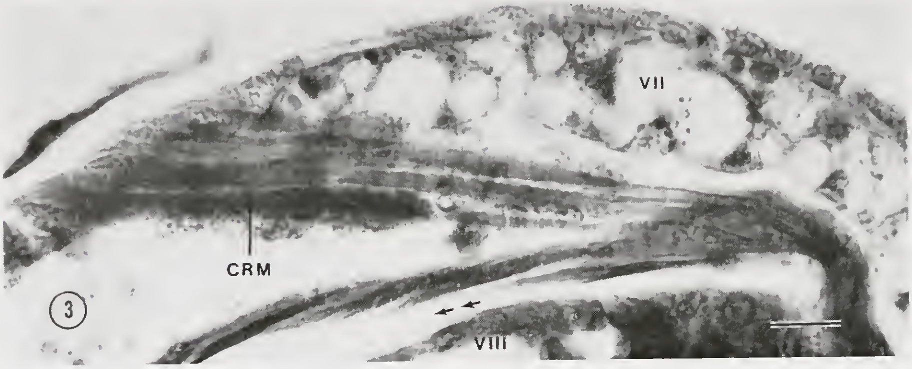
Electron microscopy shows that each of these muscles in the male is composed of 10 to 12 fibers. The muscles originate anteriorly, on the posterior lateral margins of the sclerites of abdominal segment VII. They cross the intersegmental membrane obliquely and insert on the anterior lateral margins of the sclerites of abdominal segment VIII. The origin of each muscle is on the opposite side of the body from the insertion; this results in each muscle pair being crossed.

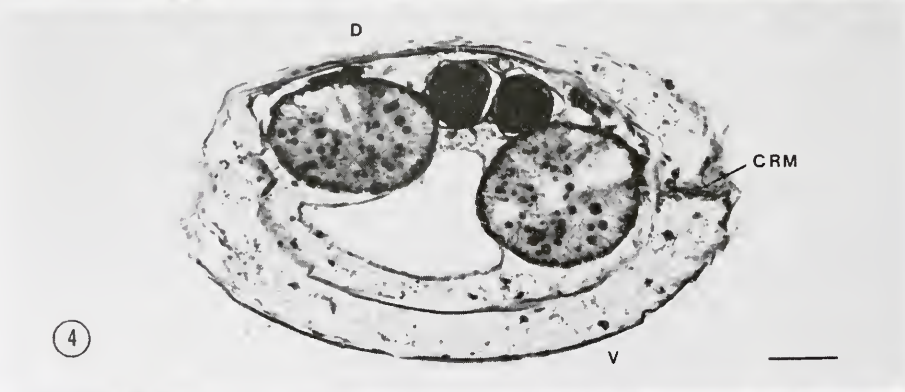
The origins of these paired muscles are quite distinct in paraffin sections, and appear localized at the posterior lateral margins of the sclerites of segment VII (Figs. 2 and 3). The insertions on segment VIII are more difficult to observe, however. This is primarily due to the close apposition of the intersegmental membrane against the sclerite cuticle of abdominal segment VII in the area where segment VIII is retracted within segment VII. The exact position of the insertions is, therefore, unclear, and they may extend to some degree circumferentially along the sclerite-intersegmental membrane junction.

Prior to the onset of rotation, both muscles of a set are of equal length. As rotation proceeds, one muscle of each set shortens and the opposed one becomes elongated (Fig. 1B). The muscles that contract remain taut, from origin to insertion, throughout the majority of the rotational process (Fig. 3). In the final 10 to 20° of rotation, bending and folding of some individual fibers occurs to a limited extent.

Upon completion of rotation, at 24 hours after emergence of the adult, both contracted and stretched muscles are present. At 48 hours, the stretched muscles can no longer be found, and only 2 to 4, rather than 10 to 12, contracted muscle fibers are present. The insertion of the contracted fibers which remain, are now




VII
CRM
③
VIII



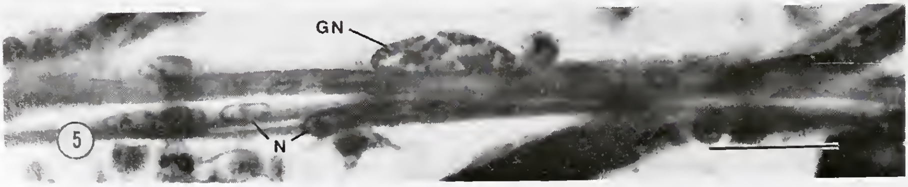


FIGURE 2. Cross section of the abdomen through the rotating region of a 26 to 27-hour pharate adult (42 hours after pupation). Compare with Figure 1A. The position of the dorsal rotational muscles is shown prior to rotation (hematoxylin). D represents dorsum

localized at the lateral, anterior margins of the sclerites of abdominal segment VIII (Figs. 1C and 4). The contracted muscles persist for at least the first week of adult life (older adults were not examined).

Characteristic of each muscle is a single giant nucleus, three to four times larger in length than the normal muscle nuclei (Figs. 5 and 6). These giant nuclei first appear in the 2 to 3-hour pharate adult (18 hours after pupation), persist until rotation is completed, and then can no longer be found.

Rotation involves an unusual reduction in the length of the contracting muscles. The muscle length is approximately 300 μm in the 26 to 27-hour pharate adult (42 hours after pupation), whereas the average length of the contracted muscle is only 69 μm in the 48-hour adult. This is equivalent to a 75 to 80% reduction during contraction. Correspondingly, the stretched muscles become elongated from 300 μm to approximately 2.5 times this length during rotation. Banding in the pre-rotational muscles is diffuse (Figs. 5 and 12), and only the A- and I-bands are apparent.

The crossed muscles are not present in the early pupa. They first appear as columns of aggregated myoblasts in the 2 to 3-hour pharate adult (18 hours after pupation); however, their position is quite unlike that in the newly-emerged adult. The insertions of the presumptive muscles are in the same general position as in the adult, near the anterior lateral margins of the eight abdominal sclerites. The myoblast columns follow the contours of the eighth abdominal sclerite to about the mid-sclerite position (tergite or sternite), whereupon they project vertically to the posterior mid-sclerite margins of the seventh abdominal segment (Fig. 6). They do not attach to the sclerites, but terminate just below the epidermis. A narrow zone of granular material, staining intensely blue with Mallory's stain (electron micrographs show this zone to consist of basement membrane), connects the myoblast columns and the basal region of the epidermal cell layer.

In the 8 to 9-hour pharate adult (24 hours after pupation), the myoblast columns have elongated and become thinner (Fig. 7). The crossed structure has become more apparent and the terminal regions (origins) beneath the epidermis of the seventh abdominal segment have begun to extend laterally.

In the 14 to 15-hour pharate adult (30 hours after pupation), the origins of the myoblast masses have begun to separate. They do so rapidly, the presumptive muscles reaching their approximate adult positions within 12 hours (Fig. 2).

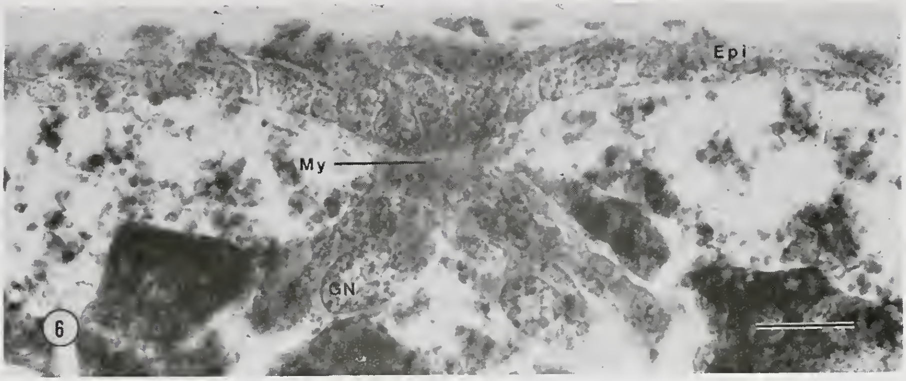
---

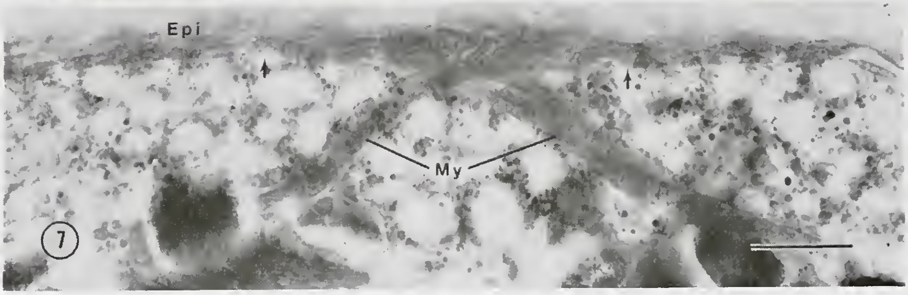
of abdomen; dRM, dorsal rotational muscles; lDV, lateral dorso-ventral muscle; V, venter of abdomen; bar equals 50 μm.

FIGURE 3. Partially contracted rotational muscle at 90° of rotation. Compare with Figure 1C. The individual fibers composing the muscle are evident. The arrows indicate the direction of rotation (Mallory's triple strain). CRM represents contracting rotational muscle; VII, abdominal segment VII; VIII, abdominal segment VIII; bar equals 20 μm.

FIGURE 4. Cross section through the abdomen of the rotating region in a 48-hour adult male, 24 hours after the completion of rotation. Compare with Figure 1D. Only one of the contracted rotational muscles is apparent in this section (hematoxylin). CRM represents contracted rotational muscle; D, dorsum of abdomen; V, venter of abdomen; bar equals 50 μm.

FIGURE 5. Fibers of the rotational muscles prior to rotation in the 26 to 27-hour pharate adult. Diffuse banding is visible in one of the fibers. The comparative sizes of the giant nucleus and the typical muscle nuclei are evident (hematoxylin). GN represents giant nucleus; N, typical muscle nuclei; bar equals 20 μm.





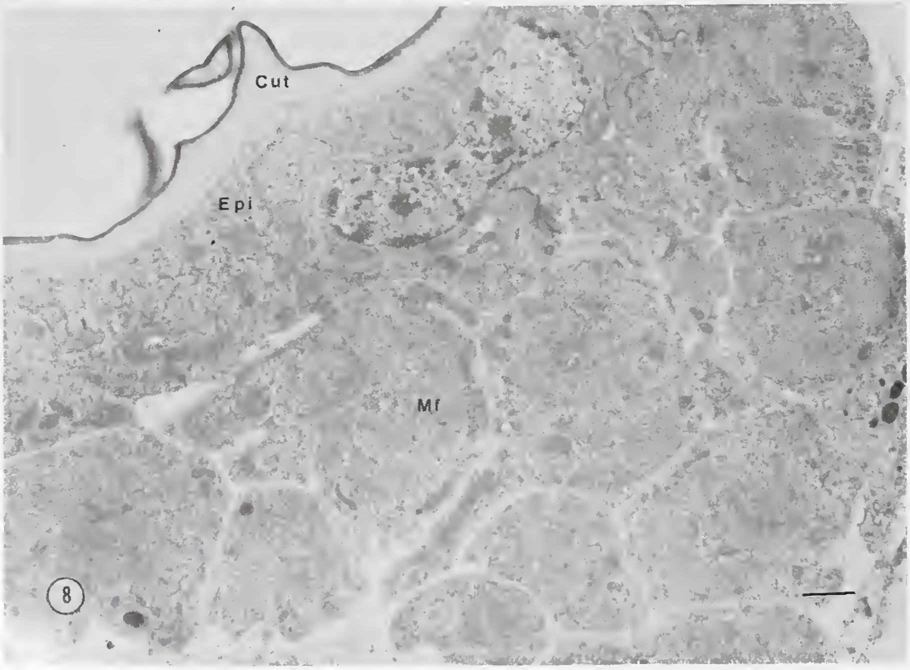


FIGURE 6. Developing rotational muscles in the 2 to 3-hour pharate adult (18 hours after pupation). The presumptive muscles appear as columns of aggregated myoblasts at this stage.

Muscle banding first becomes visible in specimens stained with hematoxylin at this time (Fig. 5).

Ultrastructure of the atypic muscles associated with rotation

The ultrastructure of the rotational muscle was examined at four stages: prior to rotation (1) in the pharate adult (42 or 48 hours after pupation), and (2) in the newly emerged adult (0 to 2 hours); (3) at 90° of rotation; and (4) at one day after rotation (48 hours post emergence).

The subdivision of each muscle into 10 to 12 fibers is evident in cross sections of the pre-rotational muscles 48 hours after pupation (Fig. 8). The muscle fibers are generally round to oval in cross section and range in diameter from 10 to 40 μm. A thin, granular basement membrane, about 22 nm thick, invests each muscle fiber. The entire fiber group, however, is not encased in a separate extracellular sheath. The fibers closely adjoin each other and the basement membranes of adjacent fibers are separated by 100 to 200 nm (Figs. 9 and 11).

The structural organization of the contractile elements can be seen in near longitudinal section in Figure 12. The muscle fibers are not separated into distinct myofibrils, and sarcoplasmic organelles appear randomly dispersed within the contractile fields. The muscle fibers are subdivided along their length into sarcomeres by Z-lines. A-bands and I-bands are the only distinguishable regions within the sarcomeres. The junction of the A- and I-bands is not clearly defined, as some of the thick filaments extend into the I-band various distances. The Z-lines are irregular and appear as discontinous patches across the muscle fibers (Figs. 12 and 16).

The sarcomere length in these muscles, prior to rotation, ranges from 6.8 to 8.3 μm. The A-band averages 3.7 μm; the I-band, 3.4 μm; and the Z-line ranges from 185 to 230 nm. These values are comparable to those of other insect slow muscles (Hagopian, 1966; Smith, Gupta and Smith, 1966; Osborne, 1967; Crossley, 1968).

The internal membrane system in these muscles is not very extensive. Invaginations of the plasma membrane to form the T-system are sparse and appear to penetrate only about a micrometer into the muscle fiber (Fig. 9). The sarcoplasmic reticulum is not abundant and dyads are uncommon. Mitochondria are sparse, and oval to slightly elongate, with well-developed cristae. They appear randomly distributed throughout the muscle fiber (Figs. 8 and 9). Numerous electron dense granules (glycogen?) are found in close association with the mitochondria.

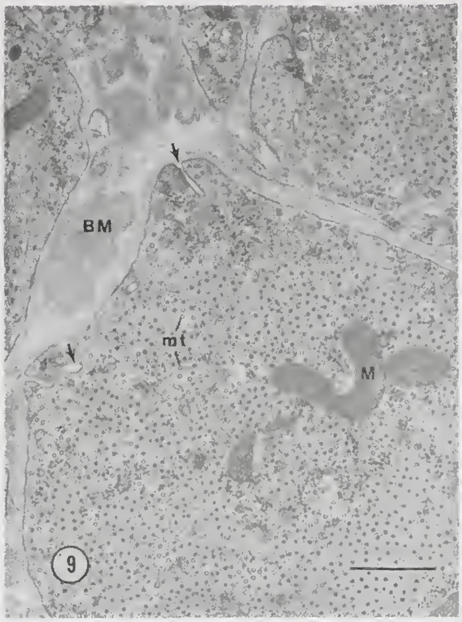
Cross sections of the muscle fibers show a thick-thin (myosin-actin) filament ratio of about 1:5 (Fig. 10), 10 to 12 thin filaments surrounding each thick fila-

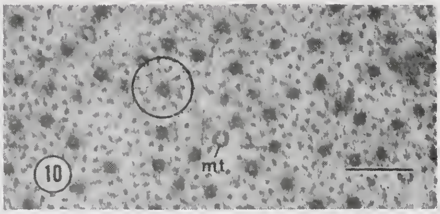
---

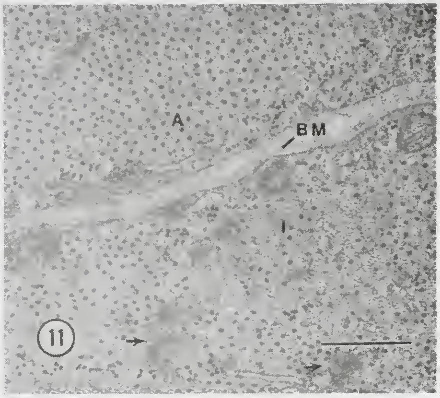
The terminal ends of the columns (origins) are not attached to the epidermis and are located along the mid-line of the presumptive 7th segment sclerites rather than at the lateral margins of the sclerites as in the newly-emerged adult (Mallory's triple stain). Epi represents epidermis; GN, giant nucleus; My, myoblast column; bar equals 20 μm.

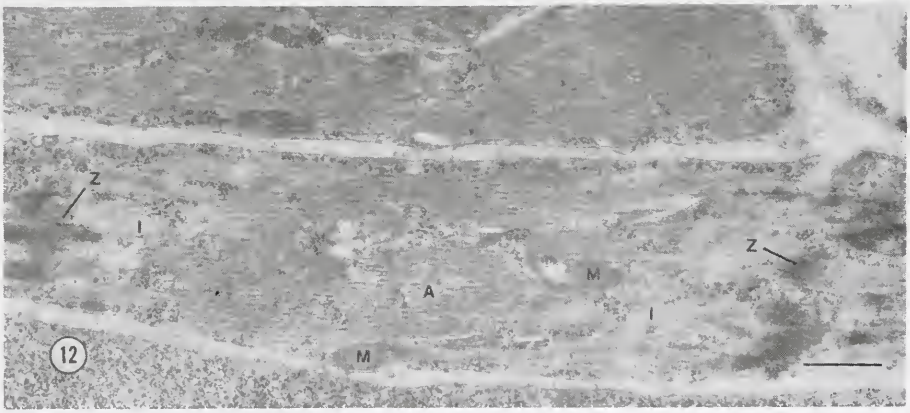
FIGURE 7. Developing rotational muscles in the 8 to 9-hour pharate adult (24 hours after pupation). The myoblast columns have elongated and become thinner, and the origins (arrows) have begun to extend laterally (Mallory's triple stain). Epi represents epidermis; My, myoblast column; bar equals 20 μm.

FIGURE 8. Cross section of a rotational muscle in the 26 to 27-hour pharate adult (42 hours after pupation). The subdivision of the muscle into 10 to 12 fibers is apparent. Cut represents cuticle; Epi, epidermis; Mf, muscle fiber; N, nucleus; bar equals 1.0 μm.









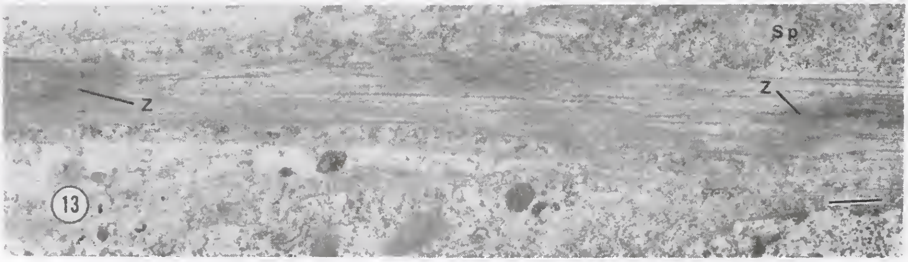


FIGURE 9. Cross section of a portion of a rotational muscle in a 26 to 27-hour pharate adult (42 hours after pupation). A thin basement membrane surrounds each muscle fiber. Invaginations of the plasmalemma (arrows) are sparse and do not penetrate deeply into the

ment. The thick filaments are 17 to 20 nm in diameter and thin filaments, 4 to 5 nm. The center-to-center spacing of the thick filaments is 55 to 60 nm. Many of the thick filaments have hollow central cores (Fig. 10). Numerous microtubules, 22 to 25 nm in diameter, are present scattered throughout the contractile fields (Fig. 10).

At 90° of rotation, three to six hours after muscle contraction is evident, one of the opposed muscles in each set is partially stretched and the other partially contracted. Longitudinal sections of a stretched fiber show a sarcomere length of 8.5 to 11.4 μm. Thick and thin filaments span the entire sarcomere, and no I-band is present (Fig. 13). The Z-lines appear more diffuse than in the pre-rotational condition and have become elongated along the axis of the muscle fiber to a width of 1.0 to 1.4 μm (Fig. 17).

Two types of contracting muscle fibers are distinguishable at the 90° rotation stage, apparently corresponding to different degrees of contraction. In one type, the sarcomere length has shortened to 3.9 to 4.5 μm,, or 45% less than in the uncontracted state. The I-band is still present (Figs. 14 and 18), and ranges from 0.18 to 0.23 μm, accounting for nearly the entire reduction in sarcomere length. All myofilaments in this fiber type parallel the long axis of the muscle. In the other type of contracting fiber (Fig. 15), the sarcomeres have an average length of 3.4 μm, or 55% less than in the uncontracted state; no I-band is present and the thick filaments penetrate to the Z-line. Many of the myofilaments are parallel to the long axis of the muscle fiber; however, some sarcomeres have their myofilaments arranged at various angles, up to 90°, to the longitudinal axis. This multidirectional arrangement of myofilaments within a muscle fiber has been previously noted by Rice (1970) in the super-contracting oesophageal muscles of the Tsetse fly, by Elder (1975) in the flight control muscle of *Vespa* and by Devine and Somolyo (1971) in vertebrate smooth muscle. The Z-banding in these two types of contracting fibers is quite different. In the unidirectional fibers (Fig. 14), the Z-lines are nearly continuous across the fibrils and are in fairly regular register. In the multidirectional fibers, most of the Z-lines are incomplete and there is poor alignment across the fibers (Fig. 15). In both types of muscle fiber, however, the Z-lines have condensed considerably from the pre-rotational condition, and are 69 to 115 nm in width (originally 185 to 230 nm).

---

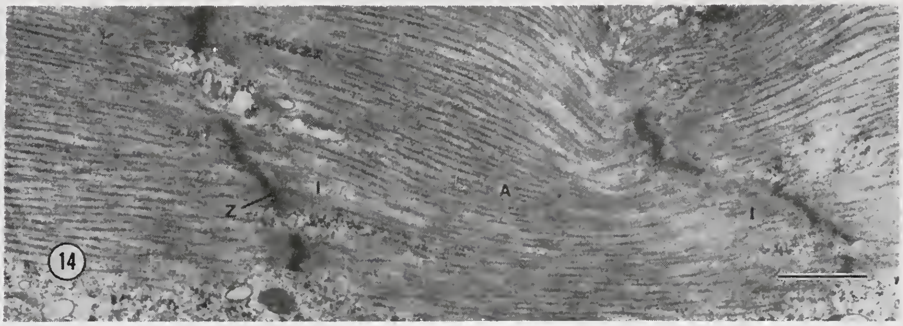
fibers. BM represents basement membrane; M, mitochondria; mt, microtubules; bar equals 0.5 μm.

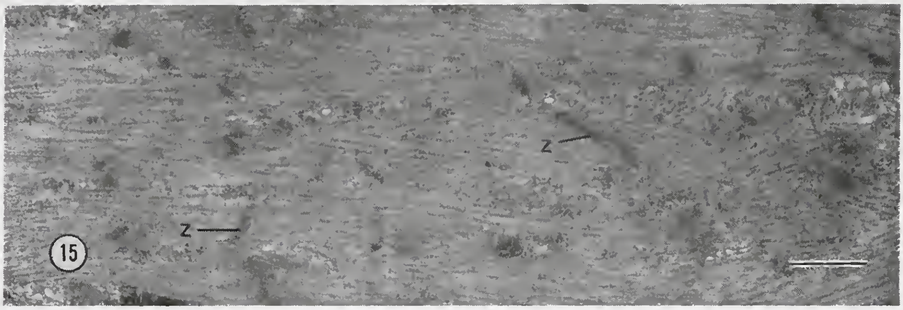
FIGURE 10. Arrangement of thick and thin filaments in a rotational muscle fiber. Each thick filament is surrounded by 10 to 12 thin filaments, maximally clear in circle. Microtubules are present in the contractile fields. Mt represents microtubules; bar equals 0.1 μm.

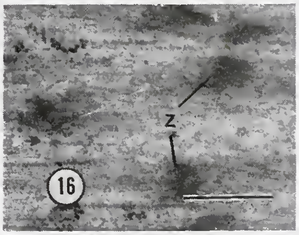
FIGURE 11. Cross section of rotational muscle fibers in a newly emerged adult. I-band regions (I), composed predominately of thin filaments, are not entirely devoid of thick filaments. Electron dense patches (arrows) are probably portions of Z-bands. A represents A-band; BM, basement membrane; I, I-band; bar equals 0.5 μm.

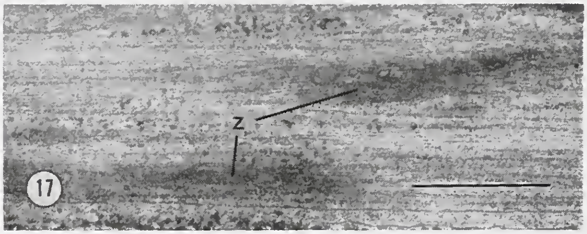
FIGURE 12. Nearly longitudinal section of rotational muscle fibers in a 32 to 33-hour pharate adult (48 hours after pupation). The irregular Z-bands (Z) limit one sarcomere. The definition between the A-band and the I-band is indistinct. Sarcoplasmic organelles appear randomly dispersed within the contractile fields. A represents A-band; I, I-band; M, mitochondria; Z, Z-band; bar equals 0.5 μm.

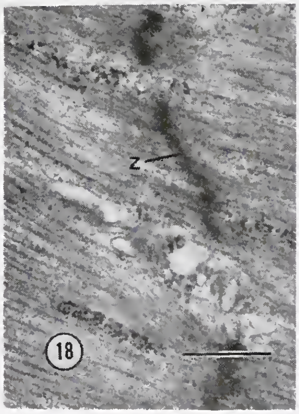
FIGURE 13. Longitudinal section of a stretched muscle fiber at 90° of rotation. The Z-bands limiting the sarcomere are elongated along the fiber axis. Thick filaments span the entire sarcomere. Sp represents sarcoplasm; Z, Z-band; bar equals 0.5 μm.











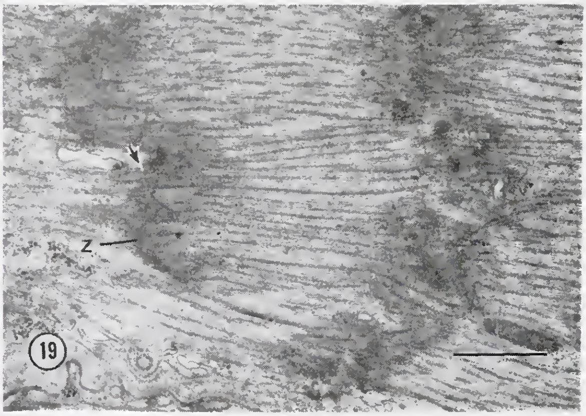


FIGURE 14. Rotational muscle contracted about 45% at 90° of rotation. A narrow I-band is still present. The Z-bands are condensed and fairly regular across the fibril. A represents A-band; I, I-band; Z, Z-band; bar equals 0.5 μm.

The partially contracted muscles appear structurally more organized in comparison to the uncontracted muscles. The appearance of the myofibrils has become somewhat more distinct and the sarcoplasmic reticulum and electron dense granules are now found principally concentrated between the myofibrils and along the Z-lines (Figs. 14 and 18). Mitochondria are generally restricted to the sarcoplasm surrounding the contractile elements and are not found within the myofilament fields.

The fully contracted muscle in the 48-hour adult has sarcomere lengths of 1.6 to 1.9 μm, a 75% reduction over that in the uncontracted muscle; this corresponds to the 75 to 80% reduction in total muscle length. There is no I-banding present and thick filaments can occasionally be seen passing through discontinuities in the Z-lines (Fig. 19). The Z-lines are less distinct than at 90° of rotation. They remain condensed, however, having a width of 69 to 90 nm. The cellular organelles are now primarily located between the myofibrils, and there is little sarcoplasm present between the sarcolemma and the contractile fields (Fig. 19). Mitochondria and electron dense granules are in close association with the Z-lines.

DISCUSSION

Two sets of opposed, crossed muscles are present in the rotating intersegmental region in the newly-emerged male adult of *A. aegypti*. These muscles were overlooked by Christophers (1922) and Hodapp (1960) in their investigations of the rotational mechanism.

The histological appearance of these muscles during terminalia rotation, while not conclusive evidence in itself, strongly suggests that they function as the driving force for rotation. The muscles are positioned to exert force in a plane nearly perpendicular to the long axis of the abdomen, the plane in which rotation occurs. The contracting muscles remain taut during rotation; they do not appear to be passively dragged along as do the longitudinal intersegmental muscles (Christophers, 1922; Hodapp, 1960). The crossed structure allows for rotation in either a clockwise or counterclockwise direction, and also probably aids in stabilizing the rotational axis along the longitudinal body axis. The contracted muscles, upon completion of rotation, are positioned to prevent further movement of the eighth segment relative to the seventh.

If the crossed muscles are solely responsible for rotation (Hodapp, 1960 and Jones, 1968, have suggested that periodic contractions of the hindgut function as

---

FIGURE 15. Rotational muscle contracted about 55% at 90° of rotation. Thick filaments span the entire sarcomere and there is no I-band present. The myofilaments do not all parallel the long axis of the muscle fiber. Z-bands are incomplete and irregular across the fibers. Z represents Z-bands; bar equals 0.5 μm.

FIGURE 16. Z-band in the pre-rotational muscle. The Z-band appears as discontinuous beads to which the thin filaments attach. Z represents Z-band; bar equals 0.5 μm.

FIGURE 17. Z-band in the stretched rotational muscle at 90° of rotation. The Z-band is diffuse and elongated. Thin filaments appear to pass through the Z-band material. Z represents z-band; bar equals 0.5 μm.

FIGURE 18. Z-band in a partially contracted muscle at 90° of rotation. The Z–bands are condensed. Z represents Z-band; bar equals 0.5 μm.

FIGURE 19. Contracted rotational muscle 24 hours after the completion of rotation. Thick filaments span the entire sarcomere and can occasionally be seen passing through discontinuities (arrow) in the Z-band. Z represents Z-band; bar equals 0.5 μm.

the driving force), then gross contraction characteristics can be inferred from movement of the terminalia during rotation. Hodapp (1960) described movement as occurring "by a series of strong, twisting motions" followed by variable quiescent periods, the one at 90° of rotation being the longest, lasting from one to six hours. These overt contraction characteristics, however, are presumably modifications produced by numerous other factors in the system. Although the muscles appear histologically functional in the pharate adult 48 hours after pupation, initial movement of the terminalia does not take place until one to four hours after adult emergence. The initial 90° of rotation requires only about three hours, whereas the final 90° requires about seventeen hours. There is a prolonged cessation of movement (contraction?) at 90° of rotation. The above characteristics of rotation are functions of the entire system and additional information is necessary to understand them. Nevertheless, with the data available, the muscles which contract appear atypic in gross physiological characteristics. They contract 75 to 80% of their original length, and this requires a period of 18 to 24 hours. In addition, these muscles display the unusual property of shortening only once. Upon the completion of rotation, the two to four contracted muscle fibers which persist appear to maintain a state of prolonged contraction.

It is interesting that the member of the muscle pair which becomes elongated (presumably stretched) never shows any conclusive evidence of contraction and soon disappears. We know of no other report of a muscle that never shortens. Whether it is never stimulated, or inhibited by the stretching, or overcome by its opponent, or affected in some other way, it degenerates and disappears without ever having shortened.

Since rotation can occur either clockwise or counterclockwise in a 1 : 1 ratio, it appears that, at random, one or the other member of a set starts to contract, or contracts more strongly, thereby determining the direction of rotation. We have no evidence as to how this is effected.

Although the rotational muscles supercontract to 75 to 80% of their original length, they possess only limited ultrastructural similarities to previously described supercontracting muscles in the Diptera (Osborne, 1967; Rice, 1970; Goldstein and Burdette, 1971; Crossley, 1968, 1972; Pringle, 1972; Elder, 1975). The rotational muscles in *A. aegypti*, especially prior to rotation, have Z-lines that appear perforated in longitudinal section. However, the characteristic cross sectional profiles of perforated Z-discs (as shown by Osborne, 1967, and Crossley, 1968) were not found. In addition, beyond 50% contraction, although a few thick filaments were found passing through discontinuities in the Z-lines of contracting fibers, this occurrence was much less than that found by other investigators. In *A. aegypti*, contraction of the rotational muscles beyond 50% appears to involve primarily a folding or overlapping of thick filaments adjacent to the Z-lines, rather than a sliding of thick filaments through perforated Z-discs.

Other ultrastructural features of the rotational muscles are typical of slow contracting muscles. The organization of these into incompletely separated myofibrils and the poor alignment of the contractile elements across the fibers are also found in other insect slow skeletal and visceral muscles (Rice, 1970; Crossley, 1968, 1972). This is in contrast to 'fast' fibrillar flight muscle in which the myofibrils are distinctly separated and the sarcomeres are in perfect register (Smith, 1966). A thick/thin filament ratio of 1 : 5 is characteristic of slow skeletal and visceral muscles, whereas rapidly contracting flight muscle has a 1 : 3 ratio. Auber (1967) has correlated a high thin/thick filament ratio with a slow work rhythm, and Goldstein (1971) has suggested that the greater number of filaments may aid in maintaining tension for prolonged periods.

The internal membrane system of muscles has been correlated with the excitation-contraction coupling and the contraction-relaxation phase in insects (Huddart and Oates, 1970; Tyrer, 1973). The poorly-developed sarcoplasmic reticulum and the low number of dyads and T-system tubules in the pre-rotational muscles in *A. aegypti* are consistent with the slow contraction rhythm of insect muscles of similar appearance.

The rotational mechanism of terminalia inversion has been reported in only a a few species of Diptera. Fittkau (1971) and Dordel (1973) have described the muscles involved in rotation in several species of chironomids, and the present study is the first report of the rotational musculature in a culicid. In the Chironomidae, rotation may be either temporary or permanent (Fittkau, 1971), and in *Clunio marinus*, a permanent 180° rotation occurs within two hours of adult emergence and involves three abdominal segments (Dordel, 1973). The two families, Chironomidae and Culicidae, are considered to be closely related phylogenetically, yet the rotational mechanism described in species of these two families is considerably different both morphologically and physiologically. Further studies of the inversion mechanism in other lower Diptera displaying a 180° rotation of the genitalia should result in interesting comparative relationships.

SUMMARY

Two sets of opposed, crossed muscles are present in the rotating region of the abdomen in male *A. aegypti*. These muscles undergo changes during rotation of the genitalia that suggest they function as the driving force for rotation. During this rotation, one muscle of each set contracts and the opposed one becomes elongated.

The contracting muscles are atypic physiologically. They contract from 300 μm to about 69 μm, and this requires a period of 18 to 24 hours. They shorten only once and those muscle fibers still present after the completion of rotation remain in a contracted condition at least for two weeks. The elongated muscles never shorten; they become stretched to approximately 2.5 times their original length and disappear soon after rotation is completed.

LITERATURE CITED

AUBER, J., 1967. Distribution of two kinds of myofilaments in insect muscles. *Amer. Zool.*, **7**: 451–456.

BARBOSA, P. and T. M. PETERS, 1969. A comparative study of egg hatching techniques for *Aedes aegypti* (L). *Mosquito News*, **29**: 548–551.

CHRISTOPHERS, S. R., 1915. The male genitalia of *Anopheles*. *Indian J. Med. Res.*, **3**: 371–394.

CHRISTOPHERS, S. R., 1922. The development and structure of the terminal abdominal segments and hypopygium of the mosquito with observations on the homologies of the terminal segments of the larvae. *Indian J. Med. Res.*, **10**: 530–572.

CRAMPTON, G. C., 1941. The external morphology of the Diptera. *Bull. Conn. Geol. Natur. Hist.*, **64**: 10–165.

Crossley, A. C., 1968. The fine structure and mechanism of breakdown of larval intersegmental muscles in the blowfly *Calliphora erythrocephala*. *J. Insect Physiol.,* **14**: 1389–1407.

Crossley, A. C., 1972. Ultrastructural changes during transition of larval to adult intersegmental muscle at metamorphosis in the blowfly *Calliphora erythrocephala* (Dipt. Calliphoridae). II. The formation of adult muscles. *J. Embryol. Exp. Morphol.,* **27**: 75–101.

Devine, C. E. and A. P. Somlyo, 1971. Thick filaments in vascular smooth muscle. *J. Cell. Biol.,* **49**: 636–649.

Dordel, H. J., 1973. Funktionsanatomische Untersuchungen über die Abdomentorsion bei der männlichen Imago von *Clunio marinus* Haliday (Diptera, Chironomidae). *Z. Morphol. Tiere,* **75**: 165–221.

Elder, H. Y., 1975. Muscle structure. Pages 1–74 in P. N. R. Usherwood, Ed., *Insect muscle,* Academic Press, London, New York.

Fittkau, E. J., 1971. Der Torsions-Mechanismus bei Chrionomiden-Hypopygium. *Limnologica,* **8**: 27–43.

Goldstein, M. A., 1971. An ultrastructural study of super-contraction in the body wall muscles of *Drosophila melanogaster* larvae. *Anat. Rec.,* **169**: 326.

Goldstein, M. A. and W. J. Burdette, 1971. Striated visceral muscle of *Drosophila melanogaster* (Dipt. Drosophilidae). *J. Morphol.,* **134**: 315–334.

Griffiths, G. C. P., 1972. *The phylogenetic classification of Diptera Cyclorrhapha.* Dr. W. Junk, N.V., The Hague, 340 pp.

Hagopian, M., 1966. The myofilament arrangement in the femoral muscle of the cockroach, *Leucophea maderae* Fabricius. *J. Cell. Biol.,* **28**: 545–562.

Hodapp, C. J., 1960. The mechanism of terminalia rotation in male *Aedes aegypti* (L). *Masters thesis, University of Maryland,* College Park, Maryland, 43 pp.

Huddart, H. and K. Oates. Ultrastructure of stick insect and locust skeletal muscle in relation to excitation-contraction coupling. *J. Insect Physiol.,* **16**: 1467–1483.

Jones, J. C., 1968. The sexual life of a mosquito. *Sci. Amer.,* **218**: 108–116.

Lee, B., 1950. Dehydration. Pages 61–62 in J. B. Gatenby and H. W. Beams, Eds., *The microtomist's vade-mecum,* 11th Edition. The Blakiston Co., Philadelphia.

Marshall, J. F., 1938. *The British mosquitoes.* W. Clowes and Sons, Ltd. London, 341 pp.

Matheson, R., 1941. *Handbook of the mosquitoes of North America.* Comstock Publishing Co. Inc., Ithaca, New York, 314 pp.

Osborne, M. P., 1967. Supercontraction in the muscles of the blowfly larva: an ultrastructural study. *J. Insect Physiol.,* **13**: 1471–1482.

Pringle, J. W. S., 1972. Arthropod muscle. Pages 491–561 in G. H. Bourne, Ed., *The structure and function of muscle,* Vol. 1, Part I, 2nd Ed. Academic Press, New York.

Rige, M. J., 1970. Supercontracting and non-supercontracting visceral muscles in the Tsetse fly, *Glossina austeni. J. Insect Physiol.,* **16**: 1109–1122.

Smith, D. S., 1966. The organization of flight muscle fibers in the Odonata. *J. Cell Biol.,* **28**: 109–126.

Smith, D. S., B. L. Gupta and U. Smith, 1966. The organization and myofilament array of insect visceral muscles. *J. Cell Sci.,* **1**: 49–57.

Tyrer, N. M., 1973. Functional development of the reticular system in an insect with synchronously differentiating cells. *J. Cell Sci.,* **12**: 197–215.

Reference: *Biol. Bull.*, **151**: 297–305. (October, 1976)

OXYGEN UPTAKE OF THE SOLITARY TUNICATE *STYELA PLICATA*

THOMAS R. FISHER

Duke University Marine Laboratory, Beaufort, North Carolina 28516; and Department of Zoology, Duke University, Durham, North Carolina 27706

The solitary tunicate, *Styela plicata*, reaches its northern-most occurrence on the east coast of North America in the vicinity of Cape Hatteras, North Carolina (Van Name, 1945). The cape is an important biogeographic boundary caused by abrupt latitudinal differences in water temperature as a result of the eastward movement of the Gulf Stream (Cerame-Vivas and Gray, 1966). Further northward colonization of *Styela* might be limited by the effects of low winter temperatures on adult survival or by summer temperatures suboptimal for reproduction (Kinne, 1963).

Just south of Cape Hatteras in the Newport River estuary, North Carolina, larval settlement of *Styela* occurs mainly during spring and fall, when water temperature is approximately 20°C; little or no settlement occurs in summer (26–30° C) or winter (5–15° C) (Sutherland and Karlson, unpublished). Similar observations on settlement of *Styela* have been made in Japanese waters (Kazihara, 1964). Since summer temperatures in excess of 20° C are common at least as far north as Cape May, New Jersey (Anon., 1954), the northern extension of *Styela plicata* may be limited by winter mortality.

In an attempt to obtain a physiological explanation of the reduced rate of larval recruitment of *Styela plicata* in the summer in the Newport River estuary, I hypothesized that the metabolic costs of routine body functions require most of the food material absorbed in the gut at the high summer temperatures (26–30° C), thus limiting reproduction, and that only during the intermediate temperatures associated with the spring and fall (20° C) was a surplus of absorbed material available for growth or reproduction.

As part of a larger study designed to test the hypothesis described above, I have measured the routine oxygen consumption of *Styela plicata* in order to estimate the metabolic costs of routine body functions. Oxygen uptake was measured as a function of body size and temperature. In addition, data on the relationship between oxygen tension and oxygen consumption were obtained to provide information on the dynamics of gas exchange in the branchial sac, the gas exchange organ of tunicates.

MATERIALS AND METHODS

At seasonally defined intervals during a two year period when water temperature had not fluctuated more than a few degrees for at least one week, specimens of *Styela plicata* were collected and transferred to an aquarium supplied with running water from the estuary. The aquarium was maintained at the temperature of the estuary at the time of collection (±1° C) and on a light-dark cycle appropriate to the time of year. Five days were allowed for adjustment to laboratory conditions, and animals were discarded at the end of one month.

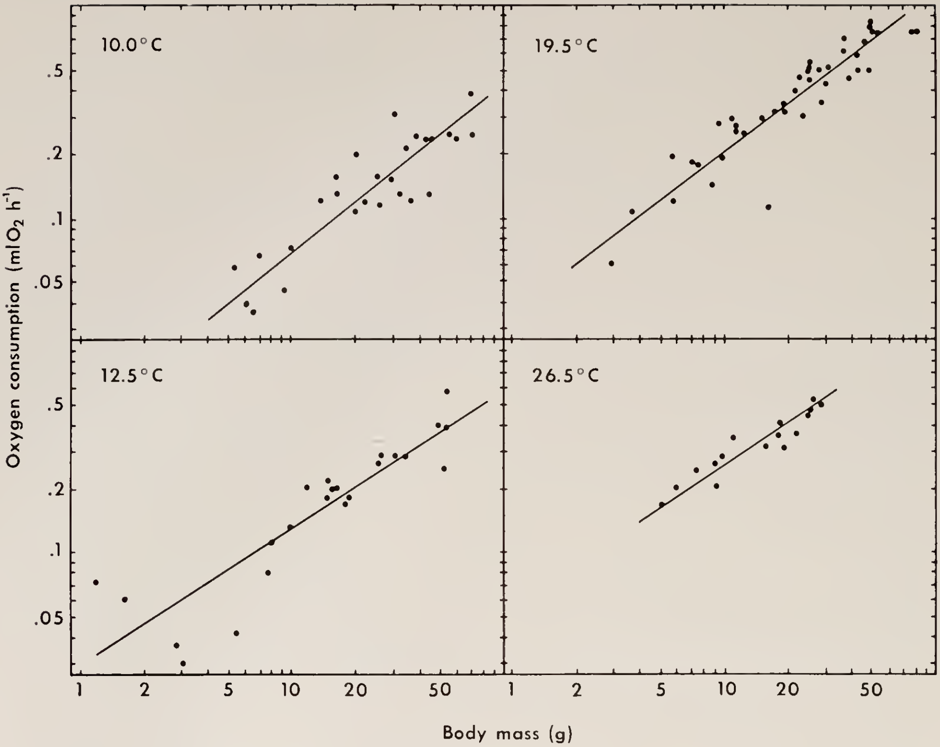


FIGURE 1 The routine oxygen consumption of *Styela plicata* as a function of body mass at four representative temperatures. The slopes of the lines are significantly greater than zero, independent of temperature, and average 0.7.

The experimental chamber for measuring respiration consisted of a 400 cc plastic beaker stoppered by a large rubber cork. Oxygen tension was measured with a YSI oxygen macro-electrode, the linearity of which was checked daily with gas standards. The polarizing circuitry was a design modified after LeFevre, Wyssbrod, and Brodsky (1970) with an output to a Beckman ten inch continuous recorder (model 1005). The oxygen electrode inserted into a hole near the bottom of the beaker, and a magnetic stirring bar maintained water flow past the electrode and provided mixing. A stainless steel screen kept the animal above the stirring magnet and prevented strong currents from developing within the chamber. Once assembled, the entire chamber was placed in a water bath maintained at the experimental temperature (±0.05° C), and recording of the oxygen tension began.

Initially, the chamber was assembled with filtered sea water (Gelman GF/A) but without an animal, and a blank rate of decrease of oxygen tension recorded. The animal was then introduced and the rate of decrease again measured. The first fifteen minutes following introduction of the animal reflected the gradual return of routine activity (as determined by siphon extension), whereupon a linear decrease in P_{O_2} began, continuing until the P_{O_2} in the chamber fell well below normoxic or air saturated conditions. At the critical P_{O_2}, (P_c), the decrease in oxygen tension became curvilinear (*i.e.*, O_2 uptake dependent on P_{O_2}), usually at a P_{O_2} well below air saturation (hypoxic conditions). The experiment was terminated when sufficient data on the rate of P_{O_2} decrease had been obtained, usually one to two hours after introduction of the animal.

The oxygen consumption ($\dot{V}_{O_2}$) of the animal was calculated from the normoxic rate of P_{O_2} decrease (estimated from the recording with a straight edge), the volume of the container, and the oxygen solubility. Measurements of $\dot{V}_{O_2}$ were taken without regard to time of day, although most occurred between 0800 and 1800 hours. Salinity of the estuarine water used in the experiments varied between 32 and 36‰.

An estimate of the error involved in determining the rate of P_{O_2} decrease was obtained by xeroxing a record and calculating $\dot{V}_{O_2}$ for each copy. The coefficient of variation ($s_x/\bar{x} \times 100$) was 5% ($\bar{x} = 0.158$ STPD ml O_2/hr, $s_x = 0.008$, $n_x = 10$; animal wet wt = 25.4 g, temperature = 10° C). An estimate of total experimental variation was obtained by using the standard deviation of the points about the calculated regression line ($s_{y/x}$) for all data at 10° C. A coefficient of variation of 11% was calculated for the above $\bar{x}$ (0.158). The difference between the two estimates is the result of systematic electrode drift during an experiment (probably very small), and variations in animal activity between experiments.

The effect of body mass (M) on oxygen consumption ($\dot{V}_{O_2}$) was analyzed by means of the allometric equation: $\log \dot{V}_{O_2} = \log K_{O_2} + b \log M$, which is equivalent to: $\dot{V}_{O_2} = K_{O_2} M^b$, where K_{O_2} is the oxygen consumption of an organism of unit mass (one gram). The scaling of metabolic rate to body size is then determined by the exponent of mass, the slope of the log-log plot (b).

RESULTS

The rate of oxygen consumption of *Styela plicata* is shown in Figure 1 at four representative temperatures and is approximately the same order of magnitude (0.1–1.0 ml O_2/hr) as that reported by Jørgensen (1952) for the tunicates *Molgula manhattensis, Ciona intestinalis,* and the bivalve *Ostrea virginica.* The slopes of the lines for data at each temperature (Table I) are significantly greater than zero ($P < 0.05$), but do not have a significant regression with temperature and average 0.7 (s.d. = 0.1). This value is not significantly different from $\frac{3}{4}$, but is significantly different from 1. Thus the intraspecific relationship between oxygen consumption and body size of *Styela plicata* is comparable to the interspecific data reviewed by Hemmingsen (1960).

The unit mass oxygen consumption [K_{O_2}, STPD ml $O_2/(\text{hr}\cdot\text{g})$] is a convenient reference point in the log-log graph of oxygen consumption versus body size. However, since K_{O_2} is well to the left of most of the data points in each of the graphs in Figure 1, it is very sensitive to the fluctuations of the slope, b, about its average value of 0.7. Therefore, the common slope, $b = 0.7$, was used to calculate K_{O_2} to reduce scatter.

Unit mass oxygen consumption, K_{O_2}, increases with temperature as shown in Figure 2. A highly significant linear regression exists between K_{O_2} and the logarithm of temperature (T): $K_{O_2} = -0.059 + 0.077 \log_{10} T$ ($r = 0.99$). The above equation may be combined with the equation relating oxygen consumption to body mass: $\dot{V}_{O_2} = (-0.059 + 0.077 \log_{10} T)\ M^{0.7}$. This combined result ex-

TABLE I

Parameters of the allometric equation $\dot{V}_{O_2} = K_{O_2}M^b$, *where* $\dot{V}_{O_2}$ *is oxygen consumption (STPD ml* O_2*/hr) of acclimatized* Styela plicata *of mass M (g),* K_{O_2} *is the oxygen consumption of an individual of unit mass [STPD ml* O_2*/(hr·g)], b is the slope of the log-log plot of the above equation, and n is the number of individuals measured.*

Temperature	K_{O_2}	b	n	K_{O_2} calculated for b = 0.7
6.0	—	—	3	0.0051
10.0	0.0230	0.556	11	0.0146
12.5	0.0293	0.649	23	0.0256
15.0	0.0275	0.751	13	0.0308
19.5	0.0348	0.769	41	0.0427
26.5	0.0536	0.670	16	0.0498
28.6	0.0567	0.829	23	0.0532
32.0	—	—	4	0.061
10/19.5	0.00859	0.879	18	0.0145
28.6/19.5	0.0560	0.747	20	0.0631

presses the predicted routine oxygen consumption as a function of body size and temperature and may be used to estimate routine metabolic costs in the testing of energetic hypotheses.

Since the scaling factor, b, does not have a significant regression with temperature, Q_{10} is independent of body size and can be calculated from the regression equation relating K_{O_2}, the unit mass oxygen consumption, to log temperature: $Q_{10} = [K_{O_2} + 10\ (dK_{O_2}/dt)]/K_{O_2} = 1 + (10/K_{O_2}) \cdot (dK_{O_2}/dt)$. This equation was used to calculate Q_{10} since detailed "R-T" information was available; it is a more accurate estimator than using $Q_{10} = (R_1/R_2)^{\frac{10}{T_1 - T_2}}$, which merely estimates the slope of the line connecting two points.

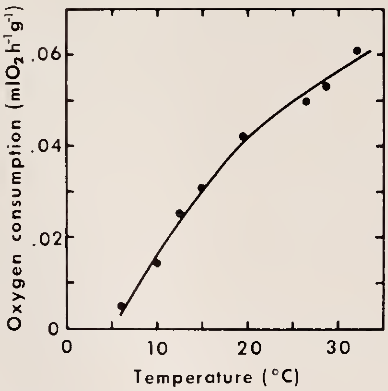


FIGURE 2. The unit mass oxygen consumption, K_{O_2}, of *Styela plicata* as a function of temperature. K_{O_2} was calculated from data such as that presented in Figure 1 for each temperature assuming a slope, b = 0.7. K_{O_2} has a significant regression with the logarithm of temperature, $K_{O_2} = -0.059 + 0.077 \log_{10}T$ (r = 0.99), and the equation is represented by the line.

TABLE II

Statistics for the relationship between critical oxygen tension, P_c, and body mass, M. P_c declines slightly with increasing body size, indicating an increasing capacity to regulate oxygen consumption.

Temperature	Equation	Correlation coefficient (4)	Significance of (r)
12.5	$P_c = 138 - 0.64\ M$	−0.98	0.99
15.0	$P_c = 129 - 0.41\ M$	−0.85	0.95
19.5	$P_c = 121 - 0.48\ M$	−0.67	0.98
18.6	$P_c = 116 - 0.69\ M$	−0.65	0.92

For the oxygen consumption of *Styela plicata* acclimatized to temperatures from 10° to 20° C, Q_{10} ranges from 5 to 2 and averages 3. However, between 20° and 30° C, Q_{10} declines to 1.5 and averages 1.7, indicating a relative temperature insensitivity and minimally increased metabolic costs for individuals acclimatized to summer temperatures.

Partial temperature independence might be expected as a result of acclimatization. To test this possibility, the oxygen consumption of *Styela* acclimatized to 19.5 ° C was measured at 28.6° C and 10° C in acute experiments with immediate transfer from 19.5° C to the experimental temperature. The transferred animals displayed a stable V_{O_2} after approximately one half hour. The data are presented in Table I. There are no significant differences between the acute and acclimatized rates at 28.6° C and 10° C, although the acute rate is 15% higher than the acclimatized rate at 28.6° C. The data suggest some acclimatization to summer temperatures (28.6° C), but the magnitude of the change is small and statistically undetectable.

The effect of oxygen tension upon oxygen uptake can be summarized by means of the critical oxyge tension, *i.e.*, the partial pressure of oxygen at which uptake becomes dependent on P_{O_2}. Larger individuals of *Styela plicata* have lower critical oxygen tensions (Table II) and therefore better regulation of oxygen consumption.

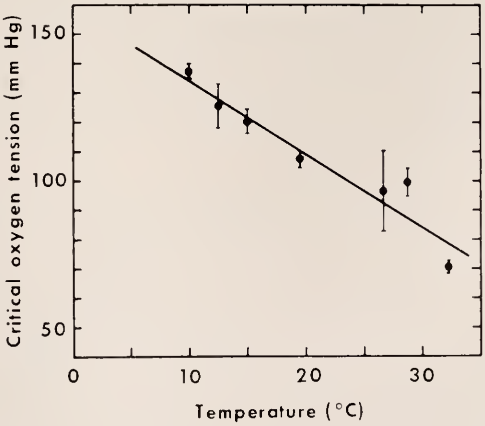


FIGURE 3. The critical oxygen tension, P_c, of *Styela plicata* as a function of temperature. The bars represent one standard deviation, and the slope of the regression equation $P_c = 157 - 2.5T$, is significantly less than zero. Above 10° C, P_c is well below air saturation.

Bayne (1971) and Taylor and Brand (1975) also report an inverse relationship between body size and critical P_{O_2} for several molluscs. The greater effect of temperature on critical oxygen tension is shown in Figure 3, which demonstrates that *Styela plicata* is a better oxygen regulator at higher temperatures.

Discussion

The hypothesis that disproportionately large maintenance costs preclude reproduction by *Styela plicata* at summer temperatures (26–30° C) is evaluated elsewhere (Fisher, 1976). The hypothesis has been shown to be inconsistent with the observed metabolic maintenance costs as indicated by oxygen consumption. Because ingestion rate and oxygen consumption show parallel scaling to body size and temperature, metabolic costs of *Styela* require a constant fraction of the resources available, equivalent to approximately 18% of the organic carbon absorbed in the gut at all temperatures. Thus, only a small and constant fraction of the carbon resources are required for maintenance, and a surplus is available for gonadal and somatic growth over the temperature range 10–30° C. During summer months predation on eggs and newly settled adults and reduction of the adult population appears to be responsible for the observed reduction in larval settlement (Fisher, 1976).

The temperature response of the oxygen consumption of *Styela* (Figure 2) reflects its geographic distribution. Between 20° and 30° C the low Q_{10} and well-developed oxygen regulatory capabilities (Fig. 3) suggest a degree of homeostasis in subtropical environments, in which *Styela* commonly occurs. In contrast, at temperatures below 10° C, *Styela plicata* becomes an oxygen conformer ($P_c \sim$ air saturation), and Q_{10} is very high (> 5). Extrapolation of the data in Figure 2 suggests that aerobic metabolism of *Styela* ceases near 5° C. However, it is likely that the temperature response of metabolic rate is sigmoid, as has been observed for other marine invertebrates (Newell, 1970). It has been possible to maintain *Styela* in the laboratory for several weeks at temperatures as low as 2–3° C, but no pumping or squirting behavior was observed. Furthermore, at temperatures below 8° C, no growth or attachment of *Styela* to its container occurred, although *Styela* grew and attached to substrate in the aquaria at all higher temperatures. These observations suggest reduced physiological adaptation of *Styela* at temperatures below 10° C. Sabbadin (1957) has reported winter mortality of *Styela plicata* in the lagoon of Venice at 4–6° C.

As discussed above, since summer temperatures suitable for reproduction occur at least as far north as Cape May, winter mortality is likely to be the major cause for the absence of *Styela* in areas north of Cape Hatteras. In the Newport River estuary, just south of Cape Hatteras, water temperature during winter months averages 10° C, and occasionally drops below 8° C during unusually cold weather, particularly at low tide. However, high tide usually brings warmer neritic water into this shallow estuary, and such low temperature conditions rarely persist for more than a few hours (temperature records, DUML museum). In contrast, winter temperatures persisting at or below 8° C are common north of Cape Hatteras and may be responsible for the absence of *Styela plicata* in these areas.

Although little mortality was observed in the running seawater aquaria, the animals maintained below 8° C showed little activity, no growth, and, in the field, would be very vulnerable to removal from substrate by waves or tidal currents. Physical removal from substrate during times of the year when there is no growth or ability to strengthen a weakened attachment is one possible cause of winter mortality in areas north of Cape Hatteras. For a soft-bodied suspension feeder such as *Styela,* removal from substrate is very likely equivalent to death. Simpson (1976) has observed that synergistic effects of several physical and biological factors limit the vertical distributions of intertidal molluscs. Similarly, it is likely that several factors together are responsible for the disappearance of *Styela plicata* at Cape Hatteras.

The capacity of an organism to regulate oxygen consumption is inversely related to the critical P_{O_2}. Physiological variables influencing the critical P_{O_2} are the gas diffusion distance, the rate of exchange of fluids on either side of the gas exchange membrane (the ventilation-perfusion ratio), and the respiratory surface area (SA) per unit volume of oxygen consumed ($SA/\dot{V}_{O_2}$). Changes in any of these will affect the critical P_{O_2}.

The inverse relationship between P_c and body size of *Styela plicata* shown in Table II can arise if gas exchange area increases faster with increasing body size than does oxygen consumption, *i.e.*, if $SA/\dot{V}_{O_2}$ increases with increasing body size. While increased ventilation/perfusion or a decreased diffusion distance can decrease P_c with increasing body size, the evidence described below suggests that increased respiratory surface area per unit volume of oxygen consumed, is at least partly responsible.

Pelseneer (1935) has reported that respiratory surface area and body size of adult molluscs form a constant ratio, which implies that the scaling factor relating respiratory surface area and body size is $b = 1$. However, the scaling factor relating oxygen consumption of molluscs to body size is less than one (Ghiretti, 1966). This suggests that the ratio of respiratory surface area to oxygen consumed increases with increasing size. von Brand, Nolan, and Mann (1948) report a constant ratio of oxygen consumption to surface area of pulmonate and operculate snails. However, surface area was estimated as $W^{2/3}$, which does not necessarily predict respiratory surface area (SA). Therefore, it appears that respiratory $SA/\dot{V}_{O_2}$ increases with increasing body size of molluscs.

Although mammalian respiratory surface area is scaled in proportion to oxygen consumption, *i.e.*, respiratory $SA/\dot{V}_{O_2}$ is constant (Tenney and Remmers, 1963), the weight dependence of respiratory $SA/\dot{V}_{O_2}$ of molluscs may be related to the dual functions of the molluscan respiratory pump: respiration and either filter-feeding or propulsion. Krogh (1951) and Wilbur and Yonge (1964) indicate that the gills of filter-feeding molluscs seem to be primarily adapted to their feeding habits, and the allometry between gill surface area and body size in molluscs may be a consequence of the physiological requirements of filter-feeding or propulsion, which apparently exceed the requirements for respiration. If this is also true for filter-feeding tunicates, then the critical oxygen tension could be expected to decline as a function of body size because of the increased respiratory surface area available for gas exchange.

The greater reduction in critical oxygen tension with increasing temperature may be the result of the increased rate of diffusion and/or a decreased diffusion distance. Since the activity of the ciliary pump in the branchial sac of *Styela plicata* can be expected to increase with temperature, a reduction in the water boundary layer associated with the branchial sac should occur. In addition to the enhanced rate of diffusion, the relationship shown in Figure 3 may reflect a diminishing diffusion distance for oxygen with increasing temperature.

Opposite effects of temperature on critical oxygen tension have been observed in fish (Hughes, 1964; Graham, 1949). Rahn (1966) has emphasized the respiratory significance of the high ventilation rates of water breathers, but the relative ventilation rates of fish are generally less than one liter per ml O_2 consumed. Many invertebrate suspension feeders such as *Styela plicata* ventilate at much higher rates, up to 20 l/ml O_2 (Jørgensen, 1966, 1975), and the higher pumping rates of suspension feeders may be responsible for the different effects of temperature on the critical oxygen tension.

This work was carried out as part of a doctoral dissertation at Duke University Marine Laboratory. Support was provided by ONR (Grant 313–3053 to J. Sutherland), Duke University, and the Society of Sigma Xi.

SUMMARY

1. The oxygen consumption of the solitary tunicate *Styela plicata* was measured in order to estimate routine metabolic maintenance costs of the animal throughout the year.

2. The acclimatized oxygen consumption of *Styela* is proportional to the 0.7 power of body weight; this value is independent of the acclimatization temperature.

3. Q_{10} declines with increasing temperature, averaging 3 between 10° and 20° C, and 1.7 between 20° and 30° C.

4. Disproportionately large metabolic costs of routine activity cannot be invoked to explain the apparent lack of reproduction by *Styela plicata* during the warmest summer months.

5. The northern limit of *Styela plicata* is in the vicinity of Cape Hatteras, North Carolina. Winter mortality of adults is likely to limit the northern extension of *Styela* beyond Hatteras, and dislodgement from substrate during cold (growth inhibited) periods is suggested as one cause of winter mortality.

6. At temperatures greater than 10° C, oxygen uptake of *Styela* is independent of oxygen tension at normoxic conditions. An analysis of the critical oxygen tension as a function of temperature and body size suggests that ciliary activity may decrease the oxygen diffusion distance in the branchial sac at increased temperatures, and that the surface area per unit volume oxygen consumed may increase with increasing body size because of the demands of filter-feeding on the branchial sac.

LITERATURE CITED

ANON., 1954. *World atlas of sea surface temperatures.* USN Hydrographic Office Publication No. 225.

BAYNE, B. L., 1971. Oxygen consumption by three species of lamellibranch molluscs in declining ambient oxygen tension. *Comp. Biochem. Physiol.,* **40A**: 955–970.

CERAME-VIVAS, M. J. AND I. E. GRAY, 1966. The distributional pattern of benthic invertebrates of the continental shelf off North Carolina. *Ecology,* **47**: 260–270.

FISHER, T. R. 1976. The cost of metabolic maintenance of suspension feeding organisms. *Mar. Biol.,* in press.

Ghiretti, F., 1966. Respiration. Pages 175–208 in K. M. Wilbur and C. M. Yonge, Eds., *Physiology of Molluscs.* Academic Press, New York.

Graham, J. M., 1949. Effects of temperature, oxygen pressure, and activity on metabolism of trout. *Can. J. Res.,* **27**: 270–288.

Hemmingsen, A. M., 1960. Energy metabolism as related to body size and respiratory surfaces, and its evolution. *Reports Steno Mem. Hosp. Nordisk,* **9(II)**: 1–110.

Hughes, G. M., 1964. Fish respiratory homeastasis. *Soc. Exp. Biol. Symp.,* **18**: 81–107.

Jørgensen, C. B., 1952. On the relationship between water transport and food requirements in some marine filter feeding invertebrates. *Biol. Bull.,* **103**: 356–363.

Jørgensen, C. B., 1966. *The biology of suspension feeding.* Pergamon Press, New York, 357 pps.

Jørgensen, C. B., 1975. Comparative physiology of suspension feeding. *Ann. Rev. Physiol.,* **37**: 57–79.

Kazihara, T., 1964. Ecological studies on marine fouling animals. *Bull. Fac. Fish Nagasaka Univ.,* **16**: 1–138.

Kinne, O., 1963. The effects of temperature and salinity on marine and brackish water animals. I. Temperature. *Oceanogr. Mar. Biol. Ann. Rev.,* **1**: 301–340.

Krogh, A., 1941. *The comparative physiology of respiratory mechanisms.* University of Pennsylvania Press, Philadelphia, 172 pp.

LeFevre, M. E., H. R. Wyssbrod, and W. A. Brodsky, 1970. Problems in the measurement of tissue respiration with the oxygen electrode. *Bioscience,* **20**: 761–764.

Newell, R. C., 1970. *Biology of intertidal animals.* Logos, London, 555 pp.

Pelseneer, P., 1935. *Essai d'ethologie zoologique d'apres l'etude des molluscs.* Acad. Roy. Belg. Classe. Sci. Publ. Fondation, Agathon de Potter, Brussels, 662 pp.

Rahn, H., 1966. Aquatic gas exchange: theory. *Resp. Physiol.,* **1**: 1–12.

Sabbadin, A., 1957. Il ciclo biologico di *Ciona intestinalis* (L.), *Molgula manhattensis* (deKay), e *Styela plicata* (Leseur) nella Laguna Veneta. *Archo. Oceanogr. Limnol.,* **11**: 1–28.

Simpson, R. D., 1976. Physical and biotic factors limiting the distribution and abundance of littoral molluscs on MacQuarie Island (sub-antarctic). *J. Exp. Mar. Biol. Ecol.,* **21**: 11–49.

Taylor, A. C. and A. R. Brand, 1975. Effects of hypoxia and body size on the oxygen consumption of the bivalve *Arctica islandica. J. Exp. Mar. Biol. Ecol.,* **19**: 187–196.

Tenney, S. M., and J. E. Remmers, 1963. Comparative quantitative morphology of the mammalian lung: diffusing area. *Nature,* **197**: 54–56.

Van Name, W. G., 1945. The North & South American Ascidians. *Amer. Mus. Natur. Hist. Bull.,* **5**: 84.

von Brand, T., M. O. Nolan, and E. R. Mann, 1948. Observations on the respiration of *Australorbis glabratus* and some other aquatic snails. *Biol. Bull.,* **95**: 199–213.

Wilbur, K. M., and C. M. Yonge, Eds., 1964. *Physiology of molluscs.* Academic Press, New York.

Reference: *Biol. Bull.*, **151**: 306–313. (October, 1976)

SEASONAL VARIATIONS OF SODIUM AND POTASSIUM CONCENTRATIONS IN DIFFERENT PARTS OF THE FROG MYOCARDIUM

T. HARZA AND LENKE MÁTYÁS

Institute of Physiology, Semmelweis University Medical School, Budapest, Hungary

Changes in environmental conditions result in alteration of the physiological parameters in amphibians to a greater extent than in higher animals. Changes in the body temperature of the frog, in different seasons, are associated with variations in the volume and electrolyte content of extracellular and intracellular fluids (Deyrup, 1964), with alteration of the function of the frog kidneys (Höber, 1927; Oliver and Shevky, 1929), and with altered responsiveness of the frog skin and kidneys to pituitrin (Hong, 1957). Schmidt-Nielsen and Forster (1954) found higher water content and increased extracellular volume in the frog under cold conditions than at room temperature. Maurer (1938) also observed increased extracellular volume in winter. Others (Churchill, Nakazawa and Drinker, 1927) found similar changes of extracellular volume in the male frog during the breeding period.

Questions arise whether these fluctuations are associated with variations in the ion content of the different tissues as well, and what is the relationship between the electrolyte concentration of these tissues and the season in which the experiment is done. Answers can be provided by statistical testing of groups of experimental data obtained in different seasons of the year regarding the two alternatives: whether the difference between any two values for ion concentrations of the tissues is due to the seasonal variation or to influence of other experimental circumstances.

The purpose of the present investigation is to determine the sodium and the potassium concentrations in various parts of the frog myocardium (sinus venosus, atrium and ventricle) in the course of a year.

Materials and Methods

The investigations of this study cover a two-year period, but, for technical reasons, no data are available for the two summer months, July and August. Female frogs of the species *Rana esculenta* were used for the experiments. The animals, weighing 30–60 g, were kept after removal from their natural environment in an open vessel with running tap water for 3–10 days prior to the experiment. Three to four hours before the experiment, the animals were transferred to a glass container with shallow water at room temperature (19–23° C).

The animals were decapitated and the spinal cord destroyed. The heart was perfused for 40–60 minutes with Ringer-solution *via* the inferior vena cava and the aorta at room temperature. The composition of the perfusate was as follows: Na^+, 113.5 mEq/l; K^+, 1.8 mEq/l; Ca^{2+}, 2.0 mEq/l; Cl^-, 114.8 mEq/l; HCO_3^-, 2.4 mEq/l; and glucose, 5.5 mmole/l. After the termination of the perfusion, hearts were separated into sinus venosus, atrium and ventricle. Each part was weighed and dissolved in concentrated nitric acid. Samples were brought to a known volume, and sodium and potassium concentrations were determined by flame photometry.

The ion concentrations found in each part of the heart were expressed as μEq/g wet weight and were plotted against the date on which the experiments had been performed. For two periods of the year, from January 1 to June 30 and from September 1 to December 31, a linear relation was calculated between the date of the experiment and the concentrations found in the different parts of the heart. Each month was considered to have 30 days, and the date of the experiment expressed as the number of elapsed days from the beginning of the series.

Since the sodium concentration is expressed as a function of date, the concentration values for each part were approximated using the formula, $f(t) = k \sin at + c$, where a is constant defining the period; k and c were then arrived at by applying the principle of the least squares.

Taking the period as one year, having 360 days, $a = \pi/180$; thus the formula takes the form $f(t) = k \sin (\pi/180)\, t + c$.

Since the minimum of the sodium concentration falls on the winter period, April 1 is to be taken as the first day of the period of the function $(t = 1)$.

If on t_i day c_i concentration value is measured, then to determine k and c one has to minimize the sum of the squares: $Q(k, c) = \Sigma_i \left(k \sin \pi/180\, t_i + c - c_i\right)$.[2]

At any rate $Q(k, c)$ does have a minimum, which can be obtained by solving the equations by partial derivatives. $\partial Q/\partial k = 0$ and $\partial Q/\partial c = 0$. Thus we have $\partial Q/\partial k = 2\, \Sigma_i \left(k \sin \pi/180\, t_i + c - c_i\right) \sin \pi/180\, t_i = 0$ and $\partial Q/\partial c = 2\, \Sigma_i \left(k \sin \pi/180\, t_i + c - c_i\right) = 0$. Hence the solution:

$$k = \frac{n \sum_i c_i \sin \frac{\pi}{180} t_i - \left(\sum_i c_i\right)\left(\sum_i \sin \frac{\pi}{180} t_i\right)}{n \sum_i \left(\sin \frac{\pi}{180} t_i\right)^2 - \left(\sum_i \sin \frac{\pi}{180} t_i\right)^2},$$

$$c = \frac{\sum_i c_i - k \sum_i \sin \frac{\pi}{180} t_i}{n}.$$

RESULTS

There is a definite sodium concentration gradient through the anatomical areas of the heart, and the potassium concentration gradient is reversed (Table I). The linear correlation coefficients between the ion concentrations and the periods of the observation are summarized in Table II. It is seen that in the first half of the year, the values for sodium concentrations are positive and highly significant in each part of the heart, whereas in the second half the values are negative and differ significantly from zero.

Potassium concentration fails to show similar variations. Except for the diminishing ventricular potassium concentration observed in the second half of the year, there is no significant correlation between potassium concentrations and the experimental periods. The "best squared" sine curve is also shown in Figures 1, 2, and 3. The value of c for the different parts of the heart is very near to appropriate averages summarized in Table I. This results from the fact that the indi-

TABLE I

Sodium and potassium concentrations in the different parts of the frog heart (μEq/g wet weight of tissues).

	Mean ± s.d. (number of experiments)	
	Na	K
Sinus venosus	106.2 ± 28.6 (57)	22.6 ± 6.1 (49)
Atrium	75.7 ± 17.4 (67)	29.2 ± 6.1 (49)
Ventricle	50.8 ± 11.3 (64)	55.7 ± 7.5 (49)

vidual experiments are dispersed nearly equally in both halves of the year, and therefore the value of $\Sigma_i \sin \pi/180\, t_i$ is very small.

The k parameter, which determines the amplitude of the function, constitutes 23.6% of the value of c in the case of the sinus venosus; it is only 15.2% for the atrium and 17.0% for the ventricle.

DISCUSSION

The sodium and potassium concentration gradients between different parts of the heart muscle observed in amphibians can be found in phylogenetically higher species (*i.e.*, in mammals), too. Holland (1960) found the sodium concentration to be 190–210 mEq/kg wet tissue in the sinoauricular node region of the rabbit heart, 130 mEq/kg in the right ventricle and 110 mEq/kg in the left ventricle, while the potassium concentration was 42 mEq/kg, 67 mEq/kg and 70 mEq/kg,

TABLE II

Correlation coefficients between sodium and potassium concentrations, and the time of the experiments.

		Na		K	
		First half of the year	Second half of the year	First half of the year	Second half of the year
Sinus venosus	r	0.54	−0.61	−0.31	−0.08
	n	36	21	31	18
	P	<0.001	<0.001	0.1 < *P* < 0.2	0.7 < *P* < 0.8
Atrium	r	0.46	−0.83	0.35	−0.38
	n	45	22	31	18
	P	<0.001	<0.001	0.05 < *P* < 0.1	0.1 < *P* < 0.2
Ventricle	r	0.60	−0.61	0.11	−0.61
	n	42	22	31	18
	P	<0.001	<0.001	0.5 < *P* < 0.6	0.001 < *P* < 0.01

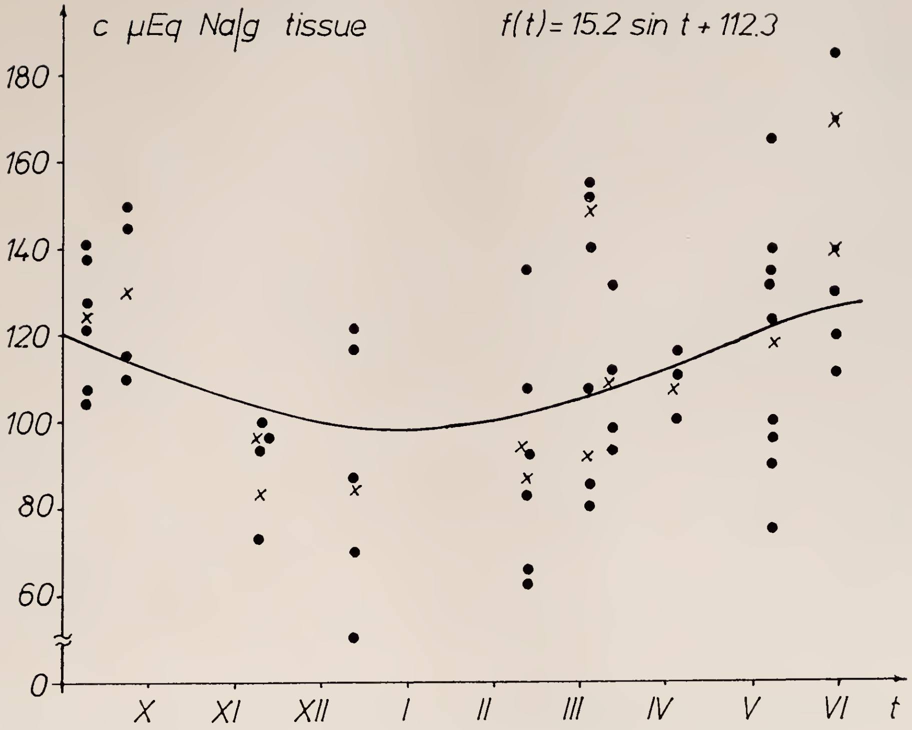


FIGURE 1. Sodium concentration of sinus venosus at different times of the year. Closed circles represent individual concentration values; crosses indicate mean values of the one-day data. A fitted sine curve is also shown.

respectively. The sodium concentration in the auricle of the human heart averaged 95.7 mEq/kg tissue, in the atrium 89.2 mEq/kg, and in the ventricle 51.3 mEq/kg. The potassium concentration, on the other hand, was 38.9 mEq/kg, 38.4 mEq/kg, and 64.8 mEq/kg, respectively (Jansen and Stappenbeck, 1962).

In previous studies (Harza, Erödi and Hársing, 1966; Harza, Váradi and Erödi, 1971) similar values for ion concentrations were reported. The values for inulin space (37.1%, 35.5% and 27.1%) and for water content (83.9%, 86.1% and 88.6%) were obtained from January 1 to April 15. By taking into account the ion concentration values obtained during this period of the year (106.0 μEq/g, 73.4 μEq/g, 45.0 μEq/g wet weight for sodium and 25.0 μEq/g, 28.0 μEq/g, 53.2 μEq/g wet weight for potassium) the intracellular ion concentrations can be estimated. The data (136, 63, 23 μEq/ml of intracellular water for sodium and 52, 54, 86 μEq/ml of intracellular water for potassium, respectively) suggest the need for another explanation of ion content values apart from the differences in the extracellular space. These findings are discussed in detail elsewhere (Harza, *et al.*, 1966; Harza *et al.*, 1971).

Correlation coefficients were calculated, after dividing the year arbitrarily into two equal periods. Sodium concentrations in all three parts of the heart increase continuously from the beginning of January to the end of June, while they decline

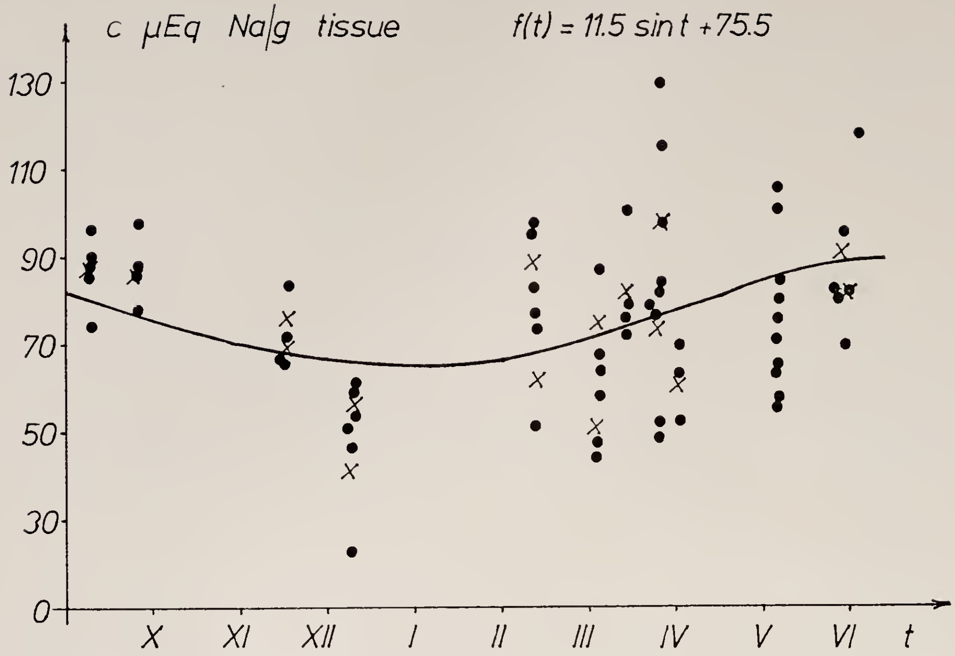


FIGURE 2. Sodium concentration of the atria at different times of the year. Closed circle represents individual concentration values; cross mark indicates mean value of the one-day data. A fitted sine curve is also shown.

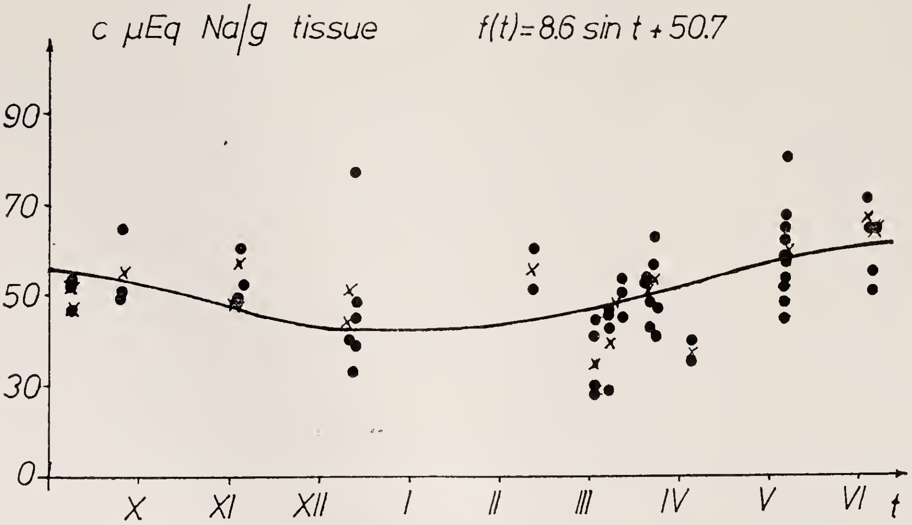


FIGURE 3. Sodium concentration of the ventricle at different times of the year. Closed circle represents individual concentration values; cross mark indicates mean value of the one-day data. A fitted sine curve is also shown.

from the beginning of September to the end of December. Therefore, the periods from January 1 to June 30 and from September 1 to December 31 were treated separately. Linear correlation coefficients can show not only the existence of correlation but can also indicate the direction of change as well. Therefore, linear correlation coefficients were calculated despite the fact that there was no evidence for an overall linearity in the "time *vs.* concentration" relationship. The deviation of the coefficients from zero in all three parts of the heart confirms the arbitrary division of the year set up by us.

No similar periodicity could be detected in potassium concentration. Therefore, the average values and standard deviation for the potassium concentration *obtained in any part* of the year can be used, given our experimental conditions.

However, the statistical treatment of the averages and standard deviations for sodium concentration does need special care for two reasons. First, standard deviation could be influenced (widened) by seasonal changes in the case of an experimental series extending over a longer period. Secondly, statistical comparison between averages, of two groups obtained at different seasons of the year, should not be carried out by applying t test. The following procedure is suggested. From the individual data for the concentrations obtained in different parts of the year, in both experimental groups, values of c can be calculated using the following equation:

$$c = \frac{\sum_i c_i - k \sum_i \frac{\pi}{180} t_i}{n}.$$

Since the value of c does not depend on the time of the experiment, a significant difference between values of c, calculated by some appropriate mathematical statistical method (*e.g.*, by applying z statistic; Dixon and Massey, 1969) from the experimental and control readings, could demonstrate the effect of the experimental procedure. (The calculation of the standard deviation is based on the equation:

$$s = \left[\frac{\sum_i [f(t_i) - c_i]^2}{n - 1}\right]^{\frac{1}{2}}.)$$

Experimental conditions (for example, removal of the animals from their natural environment prior to the experiment, not taking special care of their feeding, and the constancy in the composition of the perfusion fluid irrespective of the seasons) might raise some doubts regarding the *biological* significance of our data. However, if experimental conditions had any effect on the ion content of tissues, they did not cancel out the natural seasonal oscillations of sodium concentration. However, the lack of periodical fluctuations of potassium concentration could result from standard experimental conditions.

It is worth noting that in our earlier work we could not demonstate any changes in the inulin space and water content during a four-month experimental period.

The differential changes in sodium and potassium concentrations exclude, too, the possibility that changes in the water content are *solely* responsible for changes in cation concentration. For this and other reasons, possible fluctuations of the inulin space can not be an important factor in seasonal variation of ion content. In the sinus venosus the intracellular sodium concentration is a little higher than the extracellular one, while in the ventricle the cell water contains less sodium than the extracellular one. Therefore, sodium concentrations would have to change in opposite directions in the sinus venosus and ventricle with change in extracellular space.

However, data reflecting the seasonal fluctuations of sodium metabolism in the frog would suggest a more likely explanation. Knapowsky (personal communication, Dept. of Pathophysiology, Med. Acad., Poznan, 1973) has shown that the increased temperature results in an appreciable increase of sodium transport in the frog skin in late spring and early summer, whereas temperature has no effect on the transepithelial movement of sodium in autumn.

At lower temperatures the frog increases its body weight (Schmidt-Nielsen and Forster, 1954; Jørgensen, 1950a, 1950b). This results directly from water gain, occurring in two steps. The first, following the decrease of the temperature, is a rapid absorption, which is manifested even if the frog is kept in distilled water indicating a process independent from the ion movement. Therefore the plasma electrolyte concentration of the animals falls from 111 mM/l at room temperature to 90 mM/l at cooler temperatures (Schmidt-Nielsen and Forster, 1954). This initial, rapid water intake is followed by a slower one which is acompanied by inward movement of sodium. No net water intake could be demonstrated at higher temperatures. Disturbance of the frogs and removal from their aqueous environment results in the rapid loss of sodium and water. Excretion of the electrolyte occurs *via* the kidneys (Jørgensen, 1950a). Under these circumstances, sodium concentration in the plasma and the tissues must be lower at a lower temperature than at a higher one. Since there is no significant net transport of potassium either in or out. the result of water intake in the cold and of water loss under experimental conditions is reflected in the relative stability of the potassium concentration in the plasma and tissues.

SUMMARY

Sodium and potassium concentrations in different parts of the frog heart (sinus venosus, atrium and ventricle) were investigated at various seasons of the year. The sodium concentration showed a marked periodicity in all three parts of the heart: minimum in winter, maximum in summer. Potassium concentration showed no seasonal variations, except for reduction in potassium concentration of the ventricle in the second half of the year.

The experimental results for sodium can be fitted by a sine curve, the differences between minimum (in winter) and maximum (in summer) representing 46.4% (for sinus venosus), 30.4% (atrium) and 34.0% (ventricle) of the spring-autumn values.

Variation in the electrolyte concentration could be explained by changes in salt and water metabolism induced by fluctuations in the temperature on one hand, and by the removal of the animals from their natural environment on the other.

LITERATURE CITED

CHURCHILL, E. D., F. NAKAZAWA, AND C. K. DRINKER, 1927. The circulation of body fluids in the frog. *J. Physiol.*, **63**: 305–308.

DEYRUP, I. J., 1964. Water balance and kidney. Pages 251–328 in J. A. Moore, Ed., *Physiology of the Amphibia*. Academic Press, New York and London.

DIXON, W. J. AND F. J. MASSEY, 1969. Tests of hypotheses concerning the means of two populations. Pages 114–119 in *Introduction to statistical analysis.* McGraw-Hill, New York.

HARZA, T., J. ERÖDI AND HÁRSING, 1966. Potassium and sodium exchange in different parts of the frog heart. *Acta Physiol. Acad. Sci. Hung.,* **92**: 195–202.

HARZA, T., A. VÁRADI AND J. ERÖDI, 1971. Indiffusibilis kation fractio békaszivben. *Kisérletes Orvostudomány,* **23**: 384–390.

HÖBER, R., 1927. Neue Versuche zur Physiologie der Harnbildung. *Klin. Wschr.,* **6**: 673–676.

HOLLAND, W. C., 1960. Distribution of Na and K in various regions of isolated rabbit atria. *Amer. J. Physiol.,* **198**: 1223–1224.

HONG, S. K., 1957. Effects of Pituitrin and cold on water exchanges of frogs. *Amer. J. Physiol.,* **188**: 439–442.

JANSEN, H. M., and L. STAPPENBECK, 1962. Die regionäre Verteilung von Kalium und Natrium in Herzmuskel. *Klin. Wschr.,* **40**: 470–472.

JØRGENSEN, C. B., 1950a. Osmotic regulation in the frog, *Rana esculenta* (*L*), at low temperatures. *Acta Physiol. Scand.,* **20**: 46–55.

JØRGENSEN, C. B., 1950b. The influence of salt loss on the osmotic regulation in anurans. *Acta Physiol. Scand.,* **20**: 56–61.

MAURER, F. W., 1938. Isolation and analysis of extracellular muscle fluid in the frog. *Amer. J. Physiol.,* **124**: 456–557.

OLIVER, J., AND E. SHEVKY, 1929. A mechanism of conservation in the kidneys of the winter frog. *J. Exp. Med.,* **50**: 601–615.

SCHMIDT-NIELSEN, B., AND R. P. FORSTER, 1954. The effect of dehydration and low temperature on renal function in the bullfrog. *J. Cell. Comp. Physiol.,* **44**: 233–246.

Reference *Biol. Bull.*, **151**: 314-321. (October, 1976)

EFFECTS OF TEMPERATURE ON THE NUTRITIONAL REQUIREMENTS OF *ARTEMIA SALINA* (L.)

A. HERNANDORENA

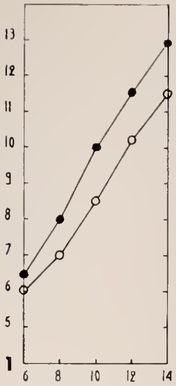
Laboratoire du Museum National d'Histoire Naturelle au Centre d'Etudes et de Recherches Scientifiques de Biarritz, 64200, France

Details concerning alterations in metabolism at the cellular and subcellular levels induced by temperature are poorly known in invertebrate animals (McWhinnie, 1967); few data exist for crustacea other than on oxygen uptake (Huggins and Munday, 1968). Even though numerous data are available for respiration rates of *Artemia* in relation to temperature (Eliassen, 1952; Grainger, 1956; Conover, 1960; Engel and Angelovic, 1968) conversion of this information into terms of food requirements and energy flow is necessary before making any conclusion (Conover, 1960). At the organismic level, the general effect of temperature is to increase growth rate. This effect has been demonstrated for different *Artemia* strains (Reeve, 1963; Hentschel, 1967; Hentig, 1971). Temperature is one of the major environmental variables which influence metabolic rate. Increasing attention is being paid to the influence of nutritional conditions and to the interactions between temperature and nutrients. Temperature has less influence on the molting frequency of starving *Balanus* when protein or lipids are used instead of carbohydrates (Barnes, Barnes and Finlayson, 1963). In *Carcinus maenas* the metabolic levels observed during starvation are related to the nature of reserves used (Wallace, 1973).

Information is lacking on how temperature affects the nutritional requirements of crustacea. The development of an artificial medium for rearing *Artemia salina* (Utah strain) by Provasoli and d'Agostino (1969) offers a good opportunity to study this problem.

MATERIALS AND METHODS

Nauplii were hatched at their rearing temperature of 25° ± 0.5° C or 30° ± 0.5° C. After hatching they were kept for 24 hours at 25° C or for 8 hours at 30° C in the hatching medium before being transfered to the nutritive media. In both cases, day one of development is 24 hours after hatching. The growth index achieved on the tenth day of development at 25° C and 30° C was used for graphical comparisons. The action of temperature on starch, adenylic acid (for adenosine monophosphate, AMP) and albumin requirements and on their relative ratios has been studied since salinity has been shown to alter the optimal starch plus AMP/albumin ratio (Hernandorena, 1974b). In the basal medium, this ratio (expressed in mg for 100 ml of media) is 100 + 60/20. The vitamin concentrations were kept constant; they are probably not limiting at 30° C since their concentrations in the basal medium are well above the minimal requirements. However, in view of House's findings (1966a), this may require further investigation. All experiments were done at 24‰ salinity.



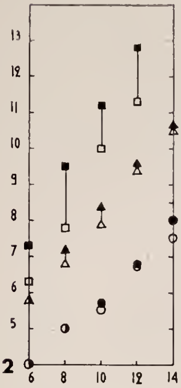
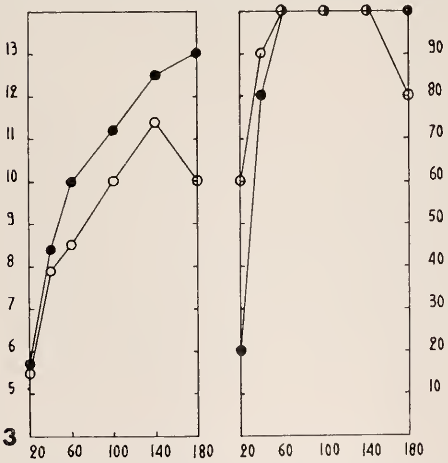


FIGURE 1. Growth rate in relation to temperature using a basal medium. Open circles indicate 25° C; closed circles, 30° C. Abscissa indicates days; ordinate, the growth index.

FIGURE 2. Growth rate in relation to AMP concentration and temperature with starch constant at 100 mg% and albumin constant at 20 mg%. Open symbols indicate 25° C; closed symbols, 30° C. Circles show AMP at 20 mg%; triangles, AMP at 40 mg%; squares, AMP at 100 mg%. Abscissa indicates days; ordinate, the growth index.

RESULTS

When using the same basal medium, the growth rate at 30° C was higher than the growth rate at 25° C (Fig. 1). These results indicate that the effect of tem-



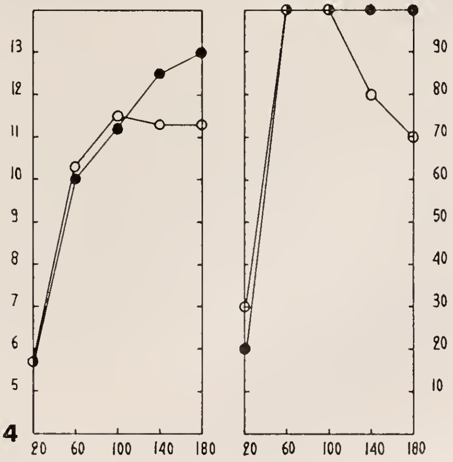
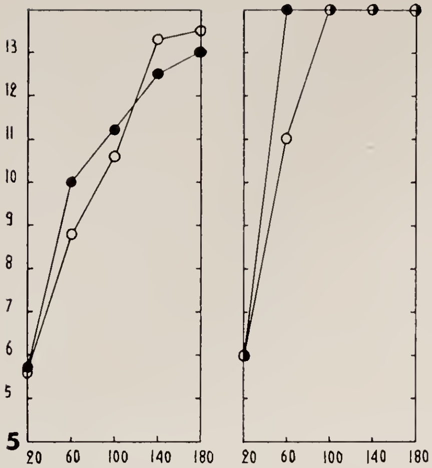
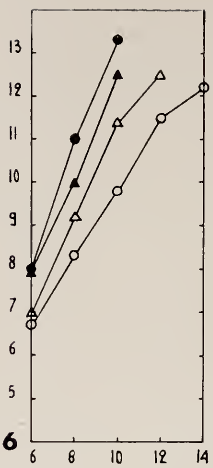


FIGURE 3. Effect of AMP requirement in relation to temperature; both abscissae represent AMP in mg%; starch constant at 100 mg%; albumin constant at 20 mg%. Open circles represent experiments at 25° C; closed circles, those at 30° C. In this and subsequent figures, growth is represented in the left-hand and survival in the right-hand graph, the left-hand ordinate being the growth index for the tenth day of development, and the right-hand ordinate being the survival percentage for index 10 (at the end of larval life).

FIGURE 4. Effect of AMP/starch ratio at 30° C; both abscissae represent AMP in mg%; albumin constant at 20 mg%. Open circles represent starch at 200 mg%; closed circles, starch at 100 mg%. The left and right ordinates are for growth and survival as in Figure 3.



5



6

FIGURE 5. Effect of AMP/albumin ratio at 30° C; both abscissae represent AMP in mg%; starch constant at 100 mg%. Open circles represent albumin at 60 mg%; closed circles, albumin at 20 mg%. The left and right ordinates are for growth and survival as in Figure 3.

FIGURE 6. Growth rate in relation to AMP/albumin ratio and temperature, with starch constant at 100 mg% and AMP constant at 140 mg%. Open symbols indicate 25° C; closed symbols, 30° C. Circles represent albumin at 60 mg%; triangles, albumin at 20 mg%. Abscissa indicates days; ordinate, the growth index.

perature depends on the available quantities and ratios of AMP, albumin and starch.

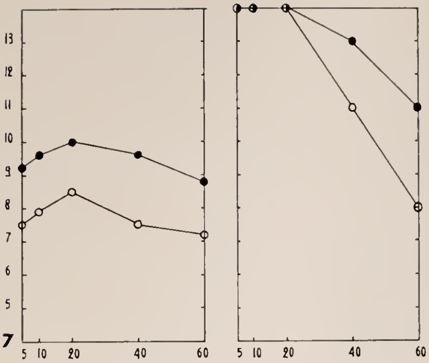
AMP requirement

With starch constant at 100 mg% and albumin constant at 20 mg, the difference between growth rate at 25° C and 30° C increased with increasing AMP concentration up to 100, mg% (Fig. 2). AMP deficiency is more detrimental at 30° C than at 25° C. Growth rate increased with increasing AMP concentration up to a 140 mg% level at 25° C and to a 180 mg% level at 30° C (Fig. 3). With increasing AMP concentration above 100 mg%, additional starch became detrimental (Fig. 4) and additional albumin beneficial (Fig. 5). The difference between growth rate at 25° C and 30° C increased with increasing AMP concentration above 100 mg% provided additional albumin was supplied (Fig. 6).

Albumin requirement

With AMP constant at 60 mg% and starch constant at 100 mg% optimal albumin concentration is 20 mg% at 25° C and 30° C, but albumin excess is less detrimental at 30° C than at 25° C (Fig. 7). At 30° C additional starch was detrimental in an albumin deficient medium and beneficial with increasing albumin concentration; however, best growth is acheived with a 100 mg% starch concentration and a 180 mg% AMP concentration, whatever the albumin concentration (Fig. 8).

7



8

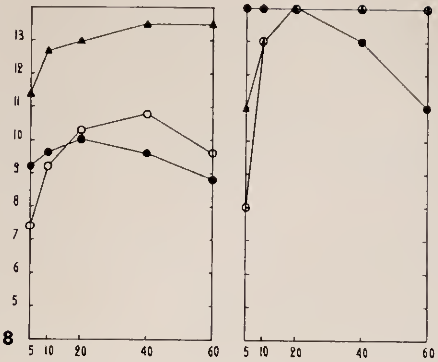


FIGURE 7. Effect of albumin requirement in relation to temperature; both abscissae represent albumin in mg%; starch constant at 100 mg%; AMP constant at 60 mg%. Open circles indicate 25° C; closed circles, 30° C; and the left and right ordinates are for growth and survival as in Figure 3.

FIGURE 8. Effects of AMP/albumin ratio and starch/albumin ratio at 30° C; both abscissae represent albumin in mg%. Open circles represent AMP at 60 mg%, starch at 200 mg%; closed circles, AMP at 60 mg%, starch at 100 mg%; and closed triangles, AMP at 180 mg%, starch at 100 mg%. Ordinates are for growth and survival as in Figure 3.

Starch requirement

With AMP constant at 60 mg% and albumin constant at 20 mg%, optimal starch concentration is 100 mg% at 25° C and less well-defined at 30° C (Fig. 9). However starch deficiency was less detrimental at 30° C than at 25° C. Additional

9

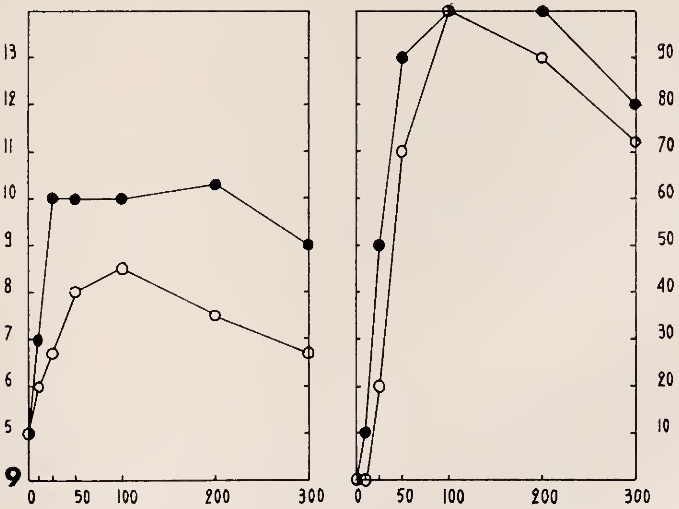
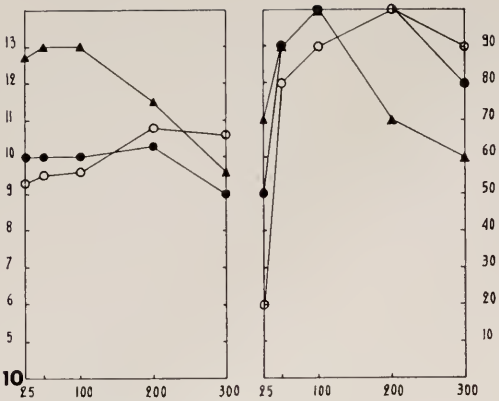
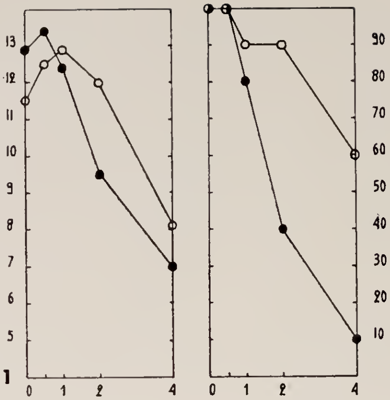


FIGURE 9. Effect of starch requirement in relation to temperatures; both abscissae represent starch in mg%; albumin constant at 20 mg%; AMP constant at 60 mg%. Open circles indicate 25° C; closed circles, 30° C. The left and right ordinates are for growth and survival as in Figure 3.



10

FIGURE 10. Effects of AMP/starch ratio and starch/albumin ratio at 30° C; both abscissae represent starch in mg%. Open circles represent AMP at 60 mg%, albumin at 40 mg%; closed circles, AMP at 60 mg%, albumin at 20 mg%; and closed triangles, AMP at 180 mg%, albumin at 20 mg%. The left and right ordinates are for growth and survival as in Figure 3.



11

FIGURE 11. Effect of lecithin requirement in relation to temperature; both abscissae represent lecithin in mg%; basal medium used. Open circles represent experiments at 25° C; closed circles, those at 30° C. The left ordinates represent growth, in this case as the index for the fourteenth day of development; the right ordinates being survival as in Figure 3.

albumin was detrimental in a starch deficient medium and beneficial with increasing starch concentration, but additional AMP was detrimental with increasing starch concentration (Fig. 10). Best growth is achieved with a 20 mg% albumin concentration and a 180 mg% AMP concentration provided additional starch was not supplied.

Lecithin requirement

With starch constant at 100 mg%, AMP constant at 60 mg% and albumin at 20 mg%, the effect of additional lecithin depends on temperature. This effect is more obvious on the fourteenth day of development (Fig. 11). Growth rate increased with increasing lecithin concentration up to a 1 mg% concentration at 25° C and up to a 0.5 mg% concentration at 30° C. Excess of lecithin is more detrimental at 30° C than at 25° C. Additional data on the effect of lecithin at 30° C will be presented elsewhere (Hernandorena, in preparation).

DISCUSSION

In *Artemia*, increasing the temperature from 25° C to 30° C resulted in a different optimal starch plus AMP/albumin ratio. At 30° C maximal growth is achieved with more AMP and less starch or more albumin than at 25° C. At 25° C and with increasing salinity, less AMP, less starch, and more albumin are required for optimal growth (Hernandorena, in preparation). The temperature-salinity relationship defined by Kinne (1963, for review) is evident during post-embryonic development in *Artemia;* that is, when salinity increases optimal temperature decreases (Hentig, 1971). Similarly, the temperature effect on the quantitative requirement for AMP is opposite to the effect of salinity, indicating that in *Artemia* the AMP requirement could be the biochemical basis for the temperature-salinity relationship.

The first conclusion to be drawn is that nutritional requirements have to be defined in relation to temperature and salinity. This emphazises the necessity for marine ecologists to start nutritional studies and to integrate the nutritional parameter.

Now the problem is to relate the temperature-induced effects on nutritional requirements to metabolism. According to Sang (1959) the balance between nutrients deserves more investigation because here one delves most closely into the examination of the metabolic processes. The information available concerning the action of temperature centers around utilization of energy producing nutrients and energy metabolism.

In insects few data are available on the influence of specific nutrients in relation to temperature. In *Periplaneta americana* the conversion efficiency (energy stored/energy input) is temperature, sex and food dependent (Prema, 1971). In *Pseudosarcophaga affinis* a nutrient balance rich in glucose was relatively beneficial in cold and detrimental in warmth (House, 1966b). The relative food value of the two synthetic diets was inverted between 15° C and 30° C. The nutrients responsible of this inversion were not defined, but the RNA content of the diets had been kept constant (House, 1972).

In *Drosophila,* temperature-dependent changes in respiration rate resulted from changes in the substrates feeding into the Krebs cycle (Burr and Hunter, 1970). Anders, Drawert, Anders and Reuther (1964) have demonstrated that the level of free amino acids is inversely related to environmental temperature in *Drosophila.* In the crayfish, low temperature acclimation resulted in measurable changes in the ratio of biochemical pathways involved in glucose utilization. Amino acid synthesis would remove intermediates from the carbohydrate cycle (McWhinnie and O'Connor, 1967).

The relative contribution of carbohydrates, lipids and proteins to energy production during the embryonic development of *Artemia* has been studied under different experimental conditions. The relative contribution of carbohydrates to energy production decreases with increasing energy drain. Lipids constitute the main energy reserve, and their utilization increases with increasing energy requirements. The contribution of proteins depends essentially on salinity (Hentig, 1971). These data concern the embryonic development which is a closed system independent of nutrition.

In *Artemia* the action of temperature must involve AMP metabolism. Temperature elevation does not increase growth rate in an AMP deficient medium. So the temperature-induced increase in growth rate is mediated through AMP metabolism. At 25° C and 24 ‰ salinity, AMP and energetic nutrient requirements increase with increasing albumin concentration in the diet (Hernandorena, 1974b). This result can be interpretated as an increased ATP requirement for protein biosynthesis since ATP produced by the energetic metabolism is necessary for amino acid incorporation into tissue proteins. Puromycin inhibition depends, in fact, on the AMP and the albumin concentrations of the diet (Hernandorena, 1975). At 30° C and 24‰ salinity, growth rate increases with increasing AMP and albumin concentration without an additional energetic nutrient requirement. Moreover, increasing starch or lecithin concentration is detrimental. It can be assumed that the relevant discrepancy between nutritional requirements at 25° C and 30° C is due to the AMP/energetic nutrient ratio.

Besides gathering metabolic data, a further object of these studies is to understand the morphogenetic significance of the dietary AMP requirement. AMP deficiency induces a supernumerary gonopode morphogenesis (Hernandorena, 1970, 1972, 1974a). It is interesting to note in view of the findings that the AMP requirement increases with temperature, and that thermal stress induced a supernumerary genital appendage in mosquitoes (Horsfall and Anderson, 1963) and one or two pairs of supernumerary appendages in *Glomeris marginata* (Juperthie-Jupeau, 1971).

Metabolic adaptations to nutritional conditions, temperature and salinity were shown to affect the quantitative AMP requirement. Hence AMP-induced morphogenesis would result from a slight shift in the balance of metabolic pathways rather than from the blocking of an essential reaction (Hernandorena, 1975).

Summary

1. The growth rate of *Artemia* in a uniform basic medium is faster at 30° C than at 25° C.

2. Temperature and salinity have opposite effects on the quantitative requirement for AMP.

3. At 30° C maximal growth is achieved with more AMP, more albumin, and less starch than at 25° C.

4. The effects of temperature are mediated by the ratio between energetic nutrients and AMP.

LITERATURE CITED

Anders, E. Von, F. Drawert, A. Anders and K. H. Reuther, 1964. Uber Kausale Zusammenhange zwischer der Zuchttemperatur dem Aminasaüren. Pool un einigen quantitativen morphologischen Phanen bei *Drosophila melanogaster*. *Z. Naturforsch.*, **196**: 495–499.

Barnes, H., M. Barnes, and D. M. Finlayson, 1963. The metabolism during starvation of *Balanus balanoides*. *J. Mar. Biol. Ass. U.K.*, **43**: 213–223.

Burr, M. J., and A. S. Hunter, 1970. Effects of temperature on *Drosophila* VII. Glutamate-aspartate transaminase activity. *Comp. Biochem. Physiol.*, **37**: 251–256.

Conover, R. J., 1960. The feeding behavior and respiration of some marine planktonic crustacea. *Biol. Bull.*, **119**: 399–415.

Eliassen, E., 1952. The energy metabolism of *Artemia salina* in relation to body size, seasonal rhythms and different salinities. *Univ. Bergen Arbok. Natuurvitensk. Rekke*, **2**: 1–18.

Engel, D. W., and J. W. Angelovic, 1968. The influence of salinity and temperature upon the respiration of brine shrimp nauplii. *Comp. Biochem. Physiol.*, **26**: 749–752.

Grainger, J. N. R., 1956. Effects of changes of temperature on the respiration of certain Crustacea. *Nature*, **178**: 930–931.

Hentig, R. Von, 1971. Einfluss von Salzgehalt und Temperatur auf Entwicklung, Wachstum, Fortpflanzung und Energiebilanz von *Artemia salina*. *Mar. Biol.*, **9**: 145–182.

Hentschel, V. E., 1967. Experimentelle Untersuchungen zum Häutungsgeschehen geschlecht sreifer Artemien (*Artemia salina* Leach, Anostraca, Crustacea). *Zool. Jahrb. Abt. Allg. Zool. Physiol. Tiere*, **73**: 336–342.

Hernandorena, A., 1970. Obtention de morphogenèses appendiculaires abortives et surnuméraires chez *Artemia salina* (L.) (Crustacé Branchiopode) par carences alimentaires de base pyrimidique et de nucléotide purique. *C. R. Hebd. Seanc. Acad. Sci. Paris,* **271**: 1406–1409.

Hernandorena, A., 1972. Signification morphogénétique du besoin alimentaire en acides nucléiques chez *Artemia salina.* II. Besoin en dérivés puriques *Arch. Zool. Exp. Gen.,* **113**: 489–498.

Hernandorena, A., 1974a. Besoin alimentaire en acide adénylique, croissance et morphogenèse d'*Artemia salina. Ann. Nutr. Aliment.,* **28**: 65–82.

Hernandorena, A., 1974b. Effects of salinity on nutritional requirements of *Artemia salina. Biol. Bull.,* **146**: 236–248.

Hernandorena, A., 1975. Metabolic significance in nucleic acid metabolism and protein synthesis of dietary AMP requirement in *Artemia salina* L. *Biol. Bull.,* **148**: 416–428.

Horsfall, W. R., and J. F. Anderson, 1963. Thermally induced genital appendages on mosquitoes. *Science,* **141**: 1183–1184.

House, H. L., 1966a. Effects and interactions of varied levels of temperature, amino acids and a vitamin on the rate of larval development in the fly *Pseudosarcophaga affinis. J. Insect Physiol.,* **12**: 1493–1501.

House, H. L., 1966b. Effect of temperature on the nutritional requirements of an insect *Pseudosarcophaga affinis* Auct. Nec. Fallen (Diptera Sarcophagidae) and its probable ecological significance. *Ann. Entomol. Soc. Amer.,* **59**: 1263–1267.

House, H. L., 1972. Inversion in the order of food superiority between temperatures effected by nutrients balance in the fly larvae *Agria housei* (Diptera: Sarcophagidae). *Can. Entomol.,* **104**: 1559–1564.

Huggins, A. K., and K. A. Munday, 1968. Crustacean metabolism. *Advan. Comp. Physiol. Biochem.,* **3**: 271–378.

Juperthie-Jupeau, L., 1971. Modification expérimentale des segments au cours du développement embryonnaire chez *Glomeris marginate* (Villers) myriapode Diplopode. *C. R. Hedb. Seanc. Acad. Sci. Paris,* **273**: 1991–1994.

Kinne, O., 1963. The effects of temperature and salinity on marine and brackish water animals. *Oceanogr. Mar. Biol. Annu. Rev.,* **1**: 301–340.

McWhinnie, M. A., 1967. *The heat responses of invertebrates (exclusive of insects) in thermobiology.* A. H. Rose Ed., Academic Press, London and New York.

McWhinnie, M. A., and J. D. O'Connor, 1967. Metabolism and low temperature acclimation in the temperate crayfish *Orconectes virilis. Comp. Biochem. Physiol.,* **20**: 131–145.

Prema, M., 1971. Energy balance in *Periplaneta americana* L. *Comp. Biochem. Physiol.,* **38A**: 449–455.

Provasoli, L., and A. S. d'Agostino, 1969. Development of artificial media for *Artemia salina. Biol. Bull.,* **136**: 434–453.

Reeve, M. R., 1963. Growth efficiency in *Artemia* under laboratory conditions. *Biol. Bull.,* **125**: 133–145.

Sang, J. H., 1959. Circumstances affecting the nutritional requirements of *Drosophila melanogaster. Ann. N. Y. Acad. Sci.,* **77**: 352–365.

Wallace, J. C., 1973. Feeding, starvation and metabolic rate in the shore crab *Carcinus maenas. Mar. Biol.,* **20**: 277–281.

Reference *Biol. Bull.*, **151**: 322–330. (October, 1976)

PERMEABILITY OF TROUT ERYTHROCYTES TO NONELECTROLYTES

F. R. HUNTER

Rocky Mountain Biological Laboratory, Crested Butte, Colorado; and the Department of Biological Sciences, University of the Pacific, Stockton, California 95211

A number of years ago M. H. Jacobs made an extensive study of the permeability of erythrocytes of a wide variety of species to various nonelectrolytes (e.g., Jacobs, 1931a, b; Jacobs, Glassman and Parpart, 1935, 1950). Among other things he was interested in the relationship between erythrocyte permeabilities and zoological classification. These studies demonstrated that each class of the vertebrates has characteristic permeabilities which are different from those of other classes. The majority of his data supported the suggestion that the more closely related two species are, the more similar are their permeabilities, but there still are distinctive differences (e.g., Jacobs, Glassman and Parpart, 1938).

The majority of Jacobs' experiments were performed measuring hemolysis times, since the use of photoelectric techniques for following volume changes had not been developed. In addition, the concept of carriers in the red cell membrane had not been well-documented when Jacobs' group was carrying on their experiments, although in their 1950 paper they suggested that some sort of special mechanism might be involved in some cases.

A. K. Solomon's group has made an extensive study of red cell permeability to nonelectrolytes. They are focusing their attention on effective pore radii rather than suggesting that carriers may be involved (*e.g.*, Owen and Solomon, 1972; Galey, Owen and Solomon, 1973; Jennings and Solomon, 1976).

In this laboratory, kinetic studies are being made of the permeability to nonelectrolytes of erythrocytes of vertebrates of the various classes in an attempt to determine the presence or absence of carriers. The present report includes a kinetic analysis, using both a swelling and a shrinking technique, of the permeability of erythrocytes of four species of trout to four nonelectrolytes. There is considerable hybridization with these fishes, but since the results were essentially the same with all of the individuals studied, this should not be a problem.

Materials and Methods

Most of the trout were caught on hook and line but a few were supplied by a hatchery. Blood was obtained by cutting the gills and heparin was the anticoagulant. In most cases the blood was used immediately but in a few instances it was stored overnight in a refrigerator with similar results. Volume changes were measured using a densimeter (cf. Mawe, 1956).

Since the swelling and shrinking experiments were performed during two different summers, there were slight differences in technique. For the swelling experiments, the temperature of the water jacket in the densimeter was maintained by circulating water by gravity at room temperature (about 18° C) which eliminated marked changes in temperature. For the shrinking experiments, water from a constant temperature water bath (20° C) was circulated through the densimeter water-jacket. A second difference was that whole blood diluted with 0.82% NaCl was used in the former series while cells washed three times in 0.82% NaCl were used in the latter. A solution of 0.82% NaCl (buffered with Tris to pH 7.5) was considered to be isotonic with the bloods (Hoar and Randall, 1969). In all of the experiments the external volume was at least 300 times the volume of cell water, and therefore the external concentration of penetrant can be considered to be constant.

Swelling experiments

For the entrance, or swelling, experiments, the method of Widdas (1954) was used. The rationale behind this method is that as one adds more and more penetrant, if simple diffusion is involved, the rate of penetration will always be proportional to the concentration gradient across the cell membrane, but if facilitated diffusion is involved, the carrier will tend to saturate and so the rate of penetration will eventually fall off. Widdas starts with the basic equation: $dS/dt = K\{(C/C + \phi) - [(S/V)/(S/V + \phi)]\}$, where S is the amount of penetrant in the cell; C, external concentration of penetrant; V, volume of cell water expressed as a fraction of the isotonic volume; ϕ, value for half-saturation of the carrier; and K, a constant. Concentrations and ϕ are expressed in isotonic units (one isotone = the isotonic concentration). If ϕ is large with respect to S and C, Widdas showed that this equation can be simplified and integrated to give; $F(C, V) = kt = C' + 1 - (1 + C)V + (1 + C) \ln [C - C'/(1 - V) \ (1 + C)]$, where C′ is the initial external concentration and k equals K/ϕ. This equation describes the kinetics of simple diffusion. If ϕ is small with respect to S and C, Widdas showed that the following equation can be derived which describes the kinetics of a system with a near-saturated carrier: $F' \ (C, V) = k't = C(1 + C)\{C' + 1 - (1 + C)V + C \ln [C - C'/(1 - V)(1 + C)\}$, where k′ equals $K\phi$.

For a given experiment, values for concentrations and volumes are known and can be substituted in the second and third equations. A table can be constructed showing the numerical values of F and F′ (C, V) for several values of V, for each value of C′ and C (cf. Widdas, 1954). Such a table has been previously published for a 1 M system (Hunter, 1968) and for an 8.1 M system (Hunter, 1970a) considering that 0.3 M nonelectrolyte is isotonic with mammalian bloods. Since the osmotic pressure of trout blood is slightly less than that of mammalian blood, the solutions used in the present study were 0.91 M thiourea in 0.82% NaCl, 7.38 M urea, ethylene glycol and glycerol in 0.82% NaCl. Since thiourea is not very soluble, it has to be used in lower concentration.

If experimental times plotted against F(C, V) fall on a single straight line and give a family of straight lines when plotted against F′(C, V), this indicates that the kinetics are those of simple diffusion. If the reverse is true, the kinetics are those of a system with a near-saturated carrier.

Details of the experimental procedure are included in the legends to the figures.

Shrinking experiments

For the exit, or shrinking experiments the method of Sen and Widdas (1962) was used. With this method, one measures the rates of exit of a penetrant into

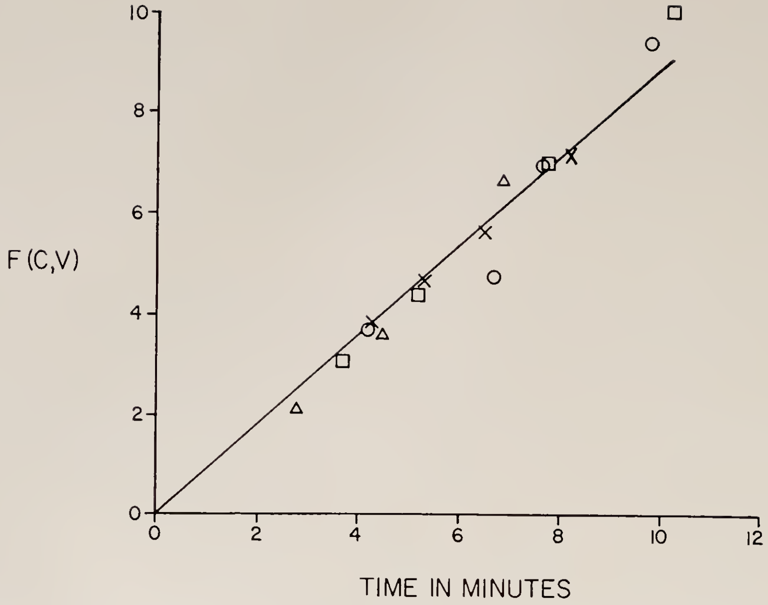


Figure 1. Experimental times plotted against F(C, V). Five additions of 0.5 ml each of 7.38 M glycerol in 0.82% NaCl were made to 8.5 ml of German brown erythrocytes in 0.82% NaCl. Additions 2–5 were recorded and are plotted. X's are for an intracellular concentration of 1.50 and an extracellular concentration of 2.84. Circles are for an intracellular concentration of 2.84 and an extracellular concentration of 4.05. Squares are for an intracellular concentration of 4.05 and an extracellular concentration of 5.14. Triangles are for an intracellular concentration of 5.14 and an extracellular concentration of 6.14. All concentrations are in isotones.

an external solution which contains increasing concentrations of the same penetrant. If ø and concentrations are small, the following relationship can be obtained: t = (Si + ø)/K (C + ø). This equation tells us that when t = 0, C = − ø.

The method for measuring *t* is given in the legend for Figure 5. Higher external concentrations were not used since the effect is not linear above 63.1 mM (Hunter, Fayad and Mayorga, 1976). Using these times, the value of ø is obtained as shown in Figure 6. It should be noted that if only simple diffusion is involved, the straight line as drawn in Figure 6 should be parallel to the x-axis giving a value of ø equal to infinity. That is to say, there is no carrier; only simple diffusion is involved.

Results

Typical series of swelling curves and shrinking curves have been published previously (Hunter, 1976). With thiourea, the volume changes resulting from the first two additions of penetrant were too small to measure accurately. With some of the other systems, the first additions of penetrant were made without recording. But in every case, from 3 to 8 swelling curves were available to analyze the kinetics of the system. Figures 1–4 illustrate typical results obtained when experimental times are plotted against either F(C, V) or F′(C, V). The results were the same

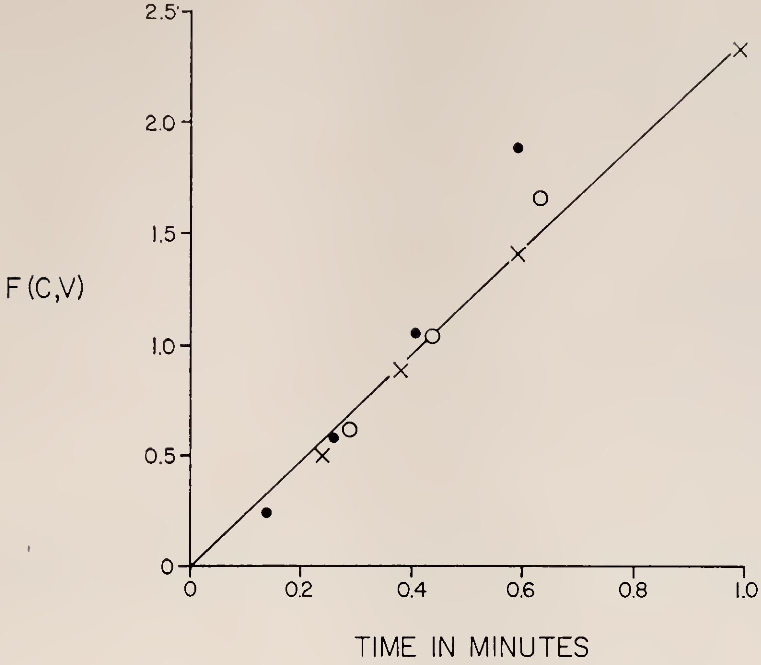


FIGURE 2. Experimental times plotted against F(C, V). Five additions of 0.1, 0.2, 0.4, 0.6 and 0.8 ml of 0.91 M thiourea in 0.82% NaCl were made to 9.9 ml of cutthroat cells in 0.82% NaCl. Only the last three additions were measured. Closed circles are for an intracellular concentration of 0.098 and an extracellular concentration of 0.220. X's are for an intracellular concentration of 0.220 and an extracellular concentration of 0.387. Open circles are for an intracellular concentration of 0.387 and an extracellular concentration of 0.583. All concentrations are in isotones.

with all four bloods and all four penetrants. In all 16 cases the experimental times when plotted against F(C, V) fell on a single straight line and in every case when these same times were plotted against F′ (C,V) a family of straight lines was obtained. In Table I are summarized relative rates of penetration of the four nonelectrolytes into erythrocytes of the four different species of trout.

Graphs were made of the initial portion of each shrinking curve (Fig. 5) in order to measure the time to reach equilibrium volume using the initial, rapid rate of exit. Average values of these times were plotted against external concentrations of penetrants (Fig. 6). These graphs should enable one to distinguish between simple and facilitated diffusion. Least squares regression lines were calculated for each of these graphs to obtain values of the x-intercepts (Table I).

DISCUSSION

Previous work has shown that glycerol, ethylene glycol, thiourea and urea cross the membrane of erythrocytes of several species of mammals and one species of birds by facilitated diffusion (Hunter, 1970a, b; Cainelli, Chui, McClure and Hunter, 1974; Hunter, 1976; Hunter, *et al.*, 1976). The present experiments

TABLE I

Average times in minutes for the erythrocytes to swell to 95% of their original volume and values of the half-saturation constant (ϕ) in millimoles calculated from the data presented in Figure 6 by the method of least squares.

Species	Nonelectrolyte							
	Glycerol		Ethylene glycol		Thiourea		Urea	
	Time	ϕ	Time	ϕ	Time	ϕ	Time	ϕ
Salvelinus fontinalis (brook trout)	5	135	0.2	−379	0.6	$\propto$	4	−646
Salmo gairdneri (rainbow trout)	6	−1,170	0.1	1,364	0.3	$\propto$	2	−1,431
Salmo trutta (German brown trout)	7	42,099	0.2	—	0.6	—	3	—
Salmo clarki (cutthroat trout)	5	−336	0.2	—	0.6	−803	4	534

were undertaken to determine whether or not carriers were involved in the penetration of these four nonelectrolytes into fish erythrocytes. The fact that experimental times obtained from the swelling curves give a single straight line when

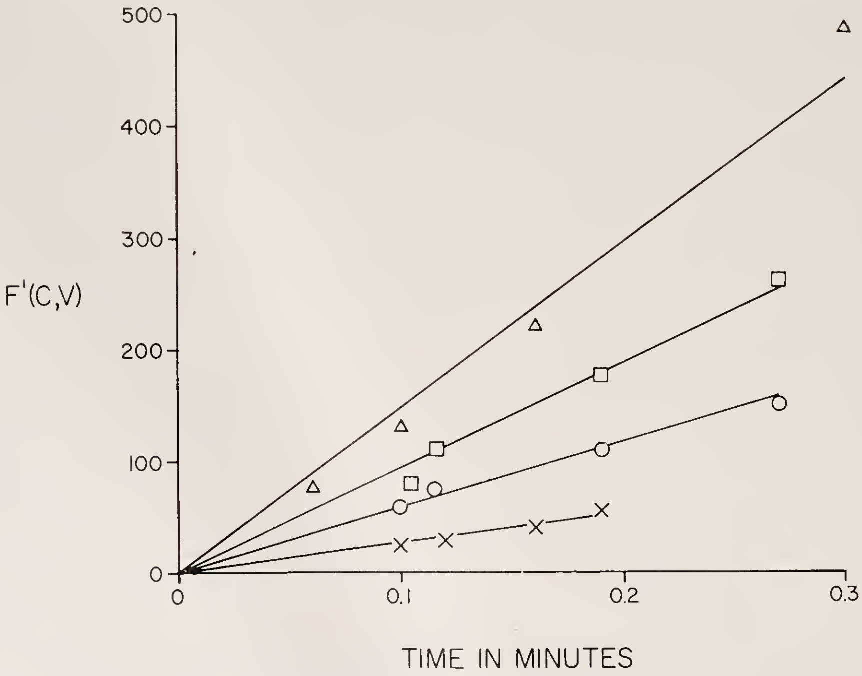


FIGURE 3. Experimental times plotted against F′(C, V) for brook trout blood and ethylene glycol penetration. The procedure was the same as for Figure 1 and the symbols have the same significance.

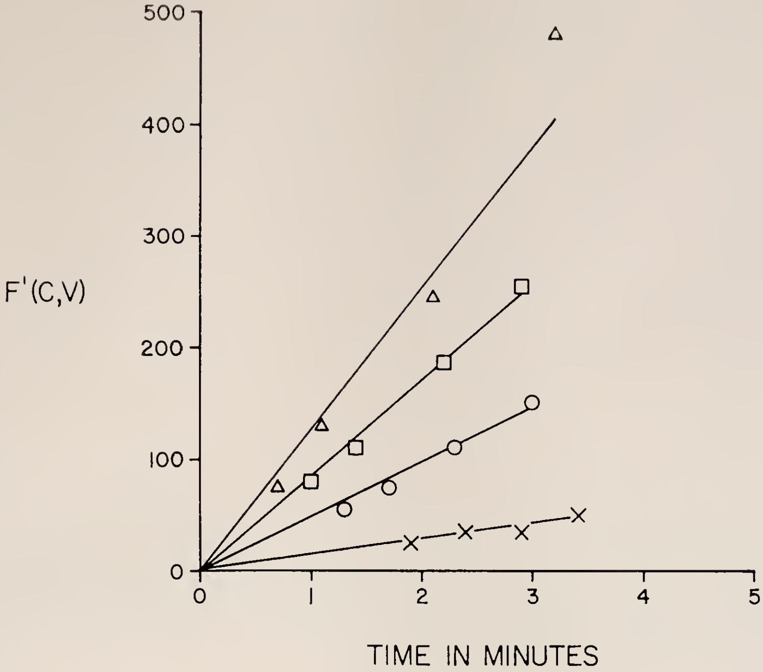


FIGURE 4. Experimental times plotted against F′(C, V) for rainbow trout and urea penetration. The procedure was the same as for Figure 3 and the symbols have the same significance.

plotted against F(C, V) indicates that there is no evidence of saturation. The family of straight lines obtained with the F′(C, V) plot is also evidence of the same thing. From the data presented in Figure 6, it can be seen that in the case of brook trout-thiourea and rainbow-thiourea the three points fall on a straight line parallel to the x-axis, predicting simple diffusion. Except for one or two of the other values in the figure, the three points in each case fall on a line almost parallel to the x-axis, suggesting simple diffusion in each case. From the calculated values for the x-intercept (Table I) it can readily be seen that most of these figures are very large (approaching infinity) and some are even negative. This means that the shrinking data, like the swelling data indicate that these four nonelectrolytes enter the erythrocytes of these four species of fish by simple diffusion (cf., Kaplan, Hays and Hays, 1974).

Comparisons of relative rates of penetration can be made using the numbers in Table I and the values in Figure 6. The absolute values are not directly comparable from the table to the figure since different concentrations of penetrants were used in the two series of experiments. Both sets of data indicate that the rates of entrance and of exit of a given nonelectrolyte are quite similar in these four closely related species. All four species have the highest permeability to ethylene glycol, then thiourea, next urea, with the lowest permeability being to glycerol. This agrees with Jacobs' data (Jacobs, 1931b, 1935).

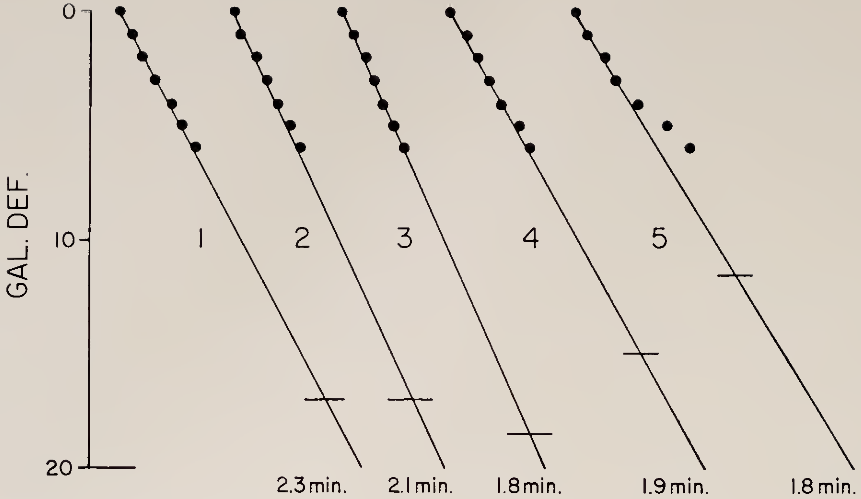


FIGURE 5. Erythrocytes of German brown trout were equilibrated in 200 mM glycerol in 0.82% NaCl (1:10 v/v). 0.25 ml of this cell suspension were added to 10 ml of 0.82% NaCl plus: 1–4.6 mM glycerol; 2–14.3 mM G; 3–23.1 mM G; 4–43.6 mM G; and 5–63.1 mM G. The initial steep portion of each curve is plotted on rectilinear coordinates. Horizontal lines represent equilibrium volume in each case. The times for the lines drawn through the initial points to intersect the horizontal lines were measured using graphs such as these. These times are indicated beside each line.

In mammalian red cells where there is a carrier for urea which also carries thiourea, relative penetration times for these two substances are reversed. Urea penetrates very rapidly and thiourea enters much more slowly. Another interesting comparison is that ethylene glycol enters the cells of the four trout species by simple diffusion much more rapidly than this molecule enters cells of some species of mammals where a carrier is involved. This might suggest that during the course of evolution, with the addition of carriers to the red cell membrane, the movement of urea was speeded up at the expense of a chemical change in the membrane which slowed down the movement of other molecules.

The author is indebted to the Faculty Research Committee of the University of the Pacific for a grant in support of this work. He also wishes to express his appreciation to Dr. Gerald E. Svendsen of Miami University for identifying the animals used in this study. Mr. Ray McDonald of the Roaring Judy Hatchery at Almont, Colorado kindly supplied the cutthroat trout used for the exit studies.

SUMMARY

Using a densimeter technique, a kinetic analysis was made, employing both entrance and exit studies, of the permeability of erythrocytes of brook trout (*Salvelinus fontinalis*), rainbow trout (*Salmo gairdneri*), German brown trout (*Salmo trutta*) and cutthroat trout (*Salmo clarki*) to glycerol, ethylene glycol, thiourea and urea. All of the data indicate that these four nonelectrolytes cross

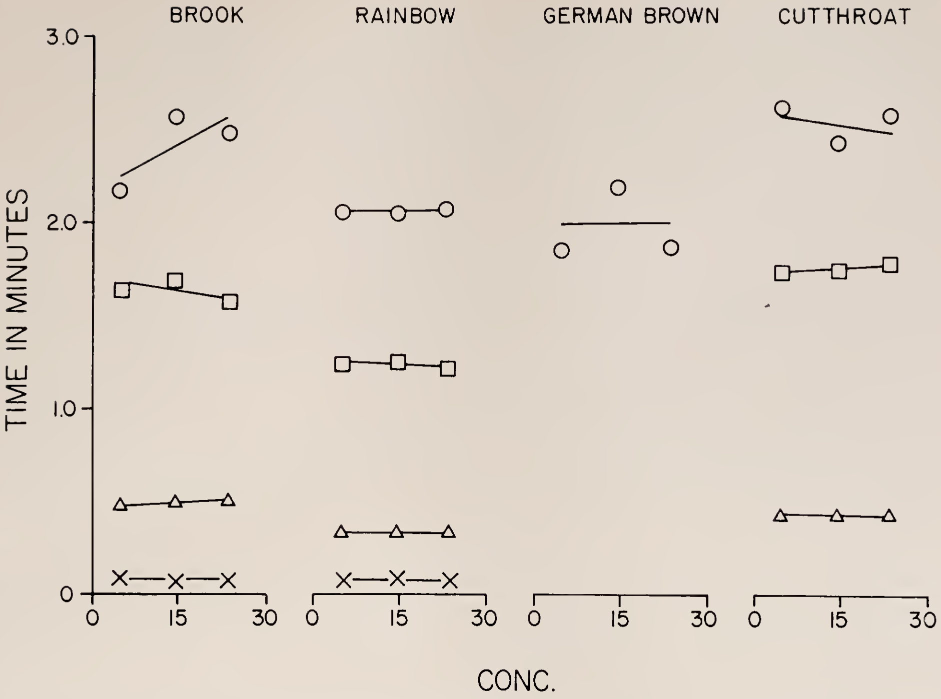


FIGURE 6. Time in minutes for initial tangent to intercept horizontal line drawn through equilibrium volume plotted against calculated external concentration of penetrant. Circles are values obtained with glycerol, X's with ethylene glycol, squares with urea and triangles with thiourea.

the membrane of the erythrocytes of these four species of fishes by simple diffusion only.

LITERATURE CITED

CAINELLI, S. R., A. CHUI, J. D. MCCLURE JR. AND F. R. HUNTER, 1974. Facilitated diffusion in erythrocytes of mammals. *Comp. Biochem. Physiol.*, **48A**: 815–825.

GALEY, W. R., J. D. OWEN AND A. K. SOLOMON, 1973. Temperature dependence of non-electrolyte permeation across red cell membranes. *J. Gen. Physiol.* **61**: 727–746.

HOAR, W. S. AND D. J. RANDALL, 1969. *Fish physiology*, Vol. I. Academic Press, New York.

HUNTER, F. R., 1968. Kinetic analysis of the permeability of human erythrocytes to NH_4Cl. *J. Gen. Physiol.*, **51**: 579–587.

HUNTER, F. R., 1970a. Facilitated diffusion in human erythrocytes. *Biochim. Biophys. Acta*, **211**: 216–222.

HUNTER, F. R., 1970b. Facilitated diffusion in pigeon erythrocytes. *Amer. J. Physiol.*, **218**: 1765–1772.

HUNTER, F. R., 1976. Facilitated diffusion in erythrocytes of additional mammals. *Comp. Biochem. Physiol.*, **53A**: in press.

HUNTER, F. R., R. FAYAD AND I. MAYORGA, 1976. Measurements of exit rates to distinguish between facilitated and simple diffusion. *Amer. J. Physiol.* **231**: in press.

JACOBS, M. H., 1931a. The permeability of the erythrocyte. *Ergeb. Biol.*, **7**: 1–55.

JACOBS, M. H., 1931b. Osmotic hemolysis and zoological classification. *Proc. Amer. Phil. Soc.*, **7**: 363–370.

Jacobs, M. H., H. N. Glassman and A. K. Parpart, 1935. Osmotic properties of the erythrocyte. VII. The temperature coefficients of certain hemolytic processes. *J. Cell. Comp. Physiol.*, **7**: 197–225.

Jacobs, M. H., H. N. Glassman and A. K. Parpart, 1938. Osmotic properties of the erythrocyte. XI. Differences in the permeability of the erythrocytes of two closely related species. *J. Cell. Comp. Physiol.*, **11**: 479–494.

Jacobs, M. H., H. N. Glassman and A. K. Parpart, 1950. Hemolysis and zoological relationship. Comparative studies with four penetrating non-electrolytes. *J. Exp. Zool.*, **113**: 277–300.

Jennings, M. L. and A. K. Solomon, 1976. Interaction between phloretin and the red blood cell membrane. *J. Gen. Physiol.*, **67**: 381–397.

Kaplan, M. A., L. Hays and R. M. Hays, 1974. Evolution of a facilitated diffusion pathway for amides in the erythrocyte. *Amer. J. Physiol.*, **226**: 1327–1334.

Mawe, R. C., 1956. The diffusion of glucose into the human red cell. *J. Cell. Comp. Physiol.*, **47**: 177–214.

Owen, J. D. and A. K. Solomon, 1972. Control of nonelectrolyte permeability in red cells. *Biochim. Biophys. Acta*, **290**: 414–418.

Sen, A. K. and W. F. Widdas, 1962. Determination of the temperature and pH dependence of glucose transfer across the human erythrocyte membrane measured by glucose exit. *J. Physiol.*, **160**: 392–403.

Widdas, W. F., 1954. Facilitated transfer of hexoses across the human erythrocyte membrane. *J. Physiol.*, **125**: 163–180.

Reference *Biol. Bull.*, **151**: 331–343. (October, 1976)

COMPARATIVE STUDIES ON THE FUNCTION OF GILLS IN SUSPENSION FEEDING BIVALVES, WITH SPECIAL REFERENCE TO EFFECTS OF SEROTONIN

C. BARKER JØRGENSEN

Zoophysiological Laboratory A, University of Copenhagen, Universitetsparken 13,
2100 Copenhagen Ø, Denmark

An extensive literature deals with the function of the exposed gill or gill fragments of suspension-feeding bivalves in transporting water and in retaining and sorting particles suspended in the water (see Jørgensen, 1966). These studies have led to the view that normal feeding results from the largely independent activities performed by the three main ciliary systems on the gill filaments: the lateral cilia transport water, the latero-frontal cirri intercept and retain particles suspended in the water, and the frontal cilia sort and transport retained particles to ciliary tracts along the gill bases or the free margins of the demibranchs.

The function of the latero-frontal cirri in straining particles from the water passing through the interfilamentar spaces and in transferring them to the frontal ciliary tracts was studied in the mussel *Mytilus edulis* by Dral (1967), who concluded that the intercirral distances, 2–3 μm, determined how small particles could be efficiently retained. Measurements of the efficiency with which *Mytilus* and other bivalves clear suspended particles from the ambient water showed, however, that even smaller particles could be efficiently retained. The discrepancy between predicted and measured efficiencies of particle retention in *Mytilus* seemed to vanish when it was observed that the cirri are featherlike structures, composed of cilia of different lengths with distal ends branching off from the main stem of the cirrus at regular intervals of about one μm (Moore, 1971). It was assumed that these side branches of the cirri form the filter that is responsible for the retention of particles smaller than the intercirral spaces (Moore, 1971; Owen, 1974a, b).

However, other suspension feeders that possess gills with short (oysters) or undeveloped (scallops) latero-frontal cirri can also efficiently retain particles (Jørgensen and Goldberg, 1953; Haven and Morales-Alamo, 1970; Vahl, 1972a, b, 1973a, b). Moreover, the *Mytilus* gill may under circumstances become leaky even to large particles despite apparently normally beating latero-frontal cirri (Jørgensen, 1975). It was, therefore, found of interest to examine more closely how exposed gills and isolated gill fragments of various gill types transport water and deal with suspended particles, and especially to compare the efficiency with which particles are retained in relation to the size and function of the latero-frontal cirri.

Serotonin (5-HT) has been found to act in a cilio-excitatory way on the bivalve gill. It has been assumed that the serotonergic nerves demonstrated in gill filaments of several bivalves control ciliary activity, especially of the lateral cilia (Aiello, 1960, 1970; Gosselin, 1961; Paparo, 1972; Paparo and Finch, 1972; Stephano and Aiello, 1975). Less is known about the role that serotonergic innervation may play in controlling the activity of the latero-frontal cirri (or other ciliary systems of the gill). It was, therefore, investigated how 5-HT affected the function of the isolated gill in transporting water and retaining suspended particles.

MATERIALS AND METHODS

The investigations were made at the Red Sea, Elat, Israel, in December 1974. The bivalves studied, all belonging to the epifauna, included *Anomia* and several species of oysters attached to anchored rafts, *Tridacna* and *Pteria* obtained from coral reefs, and one young pectinid, *Juxtamusium,* found in an oyster pond temporarily out of use.

The types of gills examined included homorhabdic, filibranch gills, *Anomia achaeus,* Gray 1849; heterorhabdic, plicate gills, *Juxtamusium maldivense* (E. A. Smith, 1903), *Pteria macroptera* (Lamarck, 1819), *Pycnodonte hyotis* (Linnaeus, 1758), and *Pycnodonte numisma* (Lamarck, 1819); and heterorhabdic, tubular gills, *Crassostrea cucullata* (von Born, 1778) = *Saccostrea cucullata* (see Ahmed, 1975), *Crassostrea lugubris* (Sowerby), *Tridacna maxima* (Röding, 1790), and *Tridacna squamosa,* Lamarck, 1819. The familial allocations of these bivalves are as follows: *Anomia* in the Anomiidae; *Juxtamusium* in the Pectinidae; *Pteria* in the Pteriidae; *Pycnodonte* in the Gryphaeidae (or in the Ostreidae); *Crassostrea* in the Ostreidae; and *Tridacna* in the Tridacnidae.

Observations on intact gills were made under a dissecting binocular microscope and on gill fragments under a monocular microscope, at magnifications up to 1000 times. Water currents produced by the gills or gill fragments were visualized by the addition of drops of a culture of the flagellate *Tetraselmis suecica* to the ambient water. The ellipsoid cells of *Tetraselmis* were about 12 × 8 μm, or more, in dimensions.

Observations started immediately after the gills had been exposed by removal of one valve and mantle. Fragments of gills were observed freshly, and at intervals, until they began to disintegrate, up to 3–4 days after they had been isolated from the bivalve. Between inspections, the preparations were placed in covered Petri dishes with sea water and kept at room temperature.

Recordings were made of gill movements and their types; movements of algal cells, and other particles suspended in water, in relation to the gill plicae and filaments, especially to what extent algae were retained by the gill or passed between the filaments; and activity of the various ciliary tracts, especially the latero-frontal cirri and the effect of 5-HT on their pattern of activity and rate of beat.

10^{-4} M 5-HT, dissolved in sea water, was usually added to the preparation to produce concentrations in the ambient medium of 10^{-5}–10^{-4} M.

RESULTS

Gill movements

Two main types of gill movements can be distinguished: first, antero-posterior and dorso-ventral, intermittent contractions, which were usually more violent (convulsive) in intact gills than in gill fragments isolated from the gill bases; and secondly, concertina-like movements of plicae or filaments (Table I). Gills exhibited the greatest variation in the convulsive type of gill movements. The most violent contractions were observed in the gill of *Pteria macroptera,* which was also

TABLE I

Types of gill movements. 0 indicates movements which are weak or absent; +, moderate; and ++, strong. The asterisk indicates gill fragments appear permanently contracted.

Species	Phasic contractions		Concertina-like movements	Nature of filament interconnections
	Untreated	After 5-HT treatment		
Anomia achaeus	+		+	ciliary bridges
Juxtamusium maldivense	++		0–+	ciliary bridges
Pteria macroptera	++	0	+	ciliary bridges
Crassostrea cucullata	0		+	epithelium with ostia
Crassostrea lugubris	0		0–+	epithelium with ostia
Pycnodonte hyotis	++	0	+	tissue bridges
P. numisma	++	0	+	tissue bridges
Tridacna maxima	+*	+*		tissue bridges
T. squamosa	+*	+*		tissue bridges

highly sensitive to mechanical stimulation, as noticed by Atkins (1936, p. 275) in *Pteria hirundo*. In other species, such as the two oysters, *Crassostrea cucullata* and *Crassostrea lugubris,* both the intact gill and isolated fragments showed only slight overall contractions, the gill movements being mainly restricted to local contractions within individual plicae.

The violent gill contractions observed especially in *P. macroptera* and both species of *Pycnodonte* were caused by contractile elements running both in an antero-posterior and dorso-ventral direction within the lamellae of the demibranchs. The elements producing the antero-posterior contractions act through the filament bridges, which are ciliary in *Pteria*. The dorso-ventral contractions are due to intrafilamentar muscle fibers.

Addition of 5-HT to the medium in concentrations of 10^{-4}–10^{-5} M in most cases caused relaxation of contracted gills, and reduced or abolished the response to mechanical stimulation. Exceptions were the two species of *Tridacna*. Fragments of the small, compact gills in these species appeared slightly contracted during the three days of observation, and the preparations showed no clear reaction to 5-HT.

The slow concertina-like movements cause neighboring plicae, or filaments, to move alternatingly towards and away from each other. In the homorhabdic, filibranch gill lamellae, as in *Anomia,* the concertina-like movements are due to the activity of the cilia constituting the ciliary bridges, which connect the filaments. In the heterohabdic, plicate gills, concertina-like movements of the plicae are caused by the activity of muscle fibers present in the abfrontal tissue of the plicae. This is also true in species in which the filaments constituting the plicae are united by ciliary bridges (*Juxtamusium, Pteria*).

The concertina-like movements of plicae were observed in all plicate gills, except in *Tridacna*. The movements tended to be intermittent and varying in intensity. In *Juxtamusium* they could be correlated with the functional state of the water-transporting lateral cilia. When all lateral cilia of a plica were active, the plica became inflated, presumably due to the hydrostatic pressure produced by the water pressed into the intraplical space by the beating lateral cilia. In this functional state, movements of the plica were discontinued. At periods of arrest of the lateral cilia the plica collapsed, and movements of the plica were often resumed. Usually intermittent periods of activity and arrest of the lateral cilia and of concertina-like movements extended over several plicae of a gill lamella.

5-HT had no clear effect on the concertina-like movements of the gill plicae in most species. In *Juxtamusium*, however, addition of 5-HT stimulated the movements.

Particle movements and water currents

Exposed gills. When one valve and mantle were removed in the specimens of bivalves examined, and a drop of *Tetraselmis* culture was added to the water, algal cells could be observed to accelerate toward the gill surface, mostly to disappear between the filaments. This behavior of suspended algae, and other particles present in the medium, indicated that currents of water moved toward the gill surface and further between the filaments.

Some algae or particles were, however, retained on the surface of the gill and were carried toward the gill base or the ventral margin of the demibranchs (Table II). In *Anomia*, all particle transport on the gill surface was toward the ventral margin, along which further transport was aborad. The exposed gill in *Pteria* also transported retained particles ventrally. In this species, however, particles that arrived at the ventral margin were not transported further, but became engaged in a stationary, rotating movement. In other species, transport of particles on the gill surface was predominantly toward the gill bases, and further orally along the dorsal groove (*Juxtamusium, Pycnodonte*). In *Tridacna*, the inner demibranch transported particles toward the ventral margin and further orally along this margin; whereas the outer, smaller, demibranch, which lacks the ventral margin and its

TABLE II

Ciliary activities and effects of 5-HT on filaments of gill fragments. For frontal cilia, v represents ventrad; d, dorsad; 0, no or weak current; +, normal current. For lateral cilia, 0 represents no or only scattered activity; (+), extensive, but unstable metachronal activity; +, stable and slow metachronal activity; ++, stable and fast metachronal activity. For latero-frontal cirri, N represents normal beating; R, beating at reduced angle; S, beating stopped; H, cirri in horizontal position; O, in oblique position; and V, in vertical position.

Species	Length of specimens in cm	Direction and strength of frontal ciliary currents		Activity of lateral cilia		Predominant patterns of activity of latero-frontal cirri	
		Ordinary filaments	Principal filaments	Untreated	After 5-HT treatment	Untreated	After 5-HT treatment
Anomia achaeus	2.5	v +	—	+	++	—	—
Juxtamusium maldivense	1.8	v +	d +	++	++	—	—
Pteria macroptera	6.0	v 0	d +	(+)–+	++	—	—
Crassostrea cucullata	Adults	v +	d +	(+)–+	++	N	SV
Crassostrea lugubris	2–3	v +	d +	(+)	++	SH-SV, N	N-SV
Pycnodonte hyotis	4–5	v 0	d +	(+)	++	SO	SO
P. numisma	3–4	v 0	d +	(+)	++	SO	SV-R
Tridacna maxima	4.0	v +	v +	(+)	+	SH-R	R-SV
T. squamosa	5.0	v +	v +	0	0	SH-SV	SH-SV

ciliary tracts, transported particles toward the inner dorsal groove (Stasek, 1962).

Gill fragments. The particle movements and water currents in relation to the gill surface could be examined in greater detail on gill fragments observed under the monocular microscope.

The predominant directions in which added *Tetraselmis* cells moved close to the frontal surface of the gill filaments are shown in Table II. In all species, except *Tridacna,* particles in interplical grooves moved dorsally, whereas on the plical crests particles in most species moved toward the ventral margin. Generally, the *Tetraselmis* cells tended to become concentrated within the interplical grooves, when these were open. Moreover, particles moved at greater speed within the grooves than on the plical crests. In some species, particles on the plical crests moved only slowly, or not at all, *e.g.,* in *Pteria* and both species of *Pycnodonte.*

Particles in the bottom of the interplical grooves travelled dorsally in a current of water produced by the frontal cilia on the principal filaments, and sometimes also on the neighboring filaments (*Crassostrea cucullata*). Presumably, the particles were predominantly suspended in the water current and not carried directly by the frontal cilia. This was indicated by the pattern of movements of the algal cells, which often crossed the principal filament moving at a level above the tips of the cilia constituting the frontal tract. Often the algae left the main current and passed through the interfilamentar spaces, (*e.g., Juxtamusium, Pteria, Pycnodonte*), or through the ostia perforating the subfilamentar membrane (*Crassostrea*).

Also particles transported toward the ventral margin higher up the plical sides and on the crests were predominantly carried in water currents produced by the frontal cilia. Mostly, particles travelled over only short distances before they disappeared between the filaments or were carried down into the interplical groove to enter the dorsally-directed water current (*e.g., Juxtamusium, Pteria, Crassostrea*).

Particles penetrating the interfilamentar space often performed characteristic jumping movements before they disappeared.

In most species, addition of 5-HT to the medium enhanced the rate at which *Tetraselmis* cells moved toward the gill surface and along the interplical grooves. Concomitantly, a greater proportion of the algae passed between filaments, and jumping before passage became more frequent and vigorous.

Jumping of particles on the surface of the gills of bivalves has previously been described by Atkins (1936, p. 275), Galtsoff (1964, p. 136), and Jørgensen (1975, p. 216). Atkins suggests that the beating of long, stout cirri is responsible for the jumping, whereas Galtsoff states that the particles are discarded by the recovery stroke of the lateral cilia. More likely the jumping reflects passive movements of the particles in local water currents produced by the activity of the metachronally beating lateral cilia (Jørgensen, 1975).

Ciliary activity

Lateral cilia. The lateral cilia are the best studied of the ciliary tracts present on the filaments of suspension-feeding bivalves. The tracts of lateral cilia vary only little between species. On gill preparations the lateral cilia beat in a plane nearly at a right angle to the long axes of the gill filaments, the active stroke being toward the abfrontal surface of the filament. The cilia beat metachronally, the metachronal wave travelling at a right angle to the plane of the beat. The direction of the effective stroke is to the left of the direction of the wave, that is, laeoplectic metachronism (Knight-Jones, 1954; Aiello and Sleigh, 1972).

The lateral cilia continued their metachronal activity on the gill fragments of all species studied, up to 4 days after preparation in *Anomia*, and 2 to 3 days in most of the other species. Often, however, the frequency of beating decreased soon after preparation, and sometimes the activity became unstable, the beat ceasing intermittently over smaller or larger distances of single filaments, or larger areas of the gill surface. Addition of 5-HT typically restored rapid and stable beating of the lateral cilia (Table II). Exceptions were *Juxtamusium* and *Tridacna*. In intact gills and gill fragments of *Juxtamusium*, the lateral cilia continued beating rapidly, with intermittent periods of ciliary arrest locally. Addition of 5-HT made no visible change in this pattern of activity. An increase in frequency of beating might have been disclosed if the frequency had been accurately measured. In *Tridacna*, the lateral cilia were only moderately active, even after addition of 5-HT in concentrations up to about 10^{-4} M.

Latero-frontal cirri. As mentioned, the latero-frontal cirri have been ascribed a special function in straining particles from the water passing through the interfilamentar spaces of the gills in suspension feeding bivalves. Special attention was, therefore, paid to the shape and function of the latero-frontal cirri. Atkins (1938) described in detail the distribution and development of latero-frontal cirri in the various types of bivalve gills, but a comparative functional study is still lacking.

Typically, latero-frontal cirri beat in a plane at a right angle to the long axis of the gill filaments. In the transitional stage between a recovery stroke and the subsequent active stroke, latero-frontal cirri occupy a horizontal position in the plane of the frontal surface of the filaments. The angle of beat is approximately 90°, so that at the end of the active stroke the cirri are oriented vertically onto the gill surface. A possible straining function of the cirri must depend upon the extent to which the cirri span over the interfilamentar space when they are in their horizontal position. It was, therefore, recorded how far into the interfilamentar space the cirri were able to reach in the various species of bivalves examined.

Latero-frontal cirri could not be distinguished on the gill filaments of *Anomia*, *Juxtamusium* and *Pteria*, even at magnifications up to 1000 times. They were present in the remaining species, but varied in size between species. Also their pattern and degree of activity, as well as their reaction to stimulation with 5-HT varied (Table II).

Length of latero-frontal cirri. The latero-frontal cirri were medium-sized in *Crassostrea cucullata* and in the two species of *Pycnodonte*, being about 17 μm in length. In the horizontal position they reached into the interfilamentar space about as far as the tips of the lateral cilia when oriented vertically on the epithelial surface during their active stroke. In the relaxed condition of the plicae this was about half the distance to the middle of the interfilamentar space.

In *C. lugubris* the latero-frontal cirri were small and inconspicuous, being about 13 μm in length. At times they were difficult to see. In their horizontal position they protruded into the interfilamentar space only to the level of the profile of the metachronal wave represented by the lateral cilia during their recovery stroke. In the relaxed plicae only a minor part of the interfilamentar space was covered by the latero-frontal cirri.

Also the two species of *Tridacna* possessed small latero-frontal cirri, about 15 μm long. In these species, however, because of the narrow interfilamentar spaces, the tips of opposing cirri met in their horizontal position. As mentioned, the plicae of the gill fragments may have remained contracted during the whole period of observation of 2–3 days. It, therefore, remains to be ascertained whether the bridging of the interfilamentar spaces by the latero-frontal cirri represents the undisturbed condition in *Tridacna*.

The above description of the relations of the latero-frontal cirri to the interfilamentar space applies to the ordinary filaments in all species examined. It also applies to the principal filaments, except in the two species of *Pycnodonte* and in *C. lugubris*. In these the latero-frontal cirri on the principal filaments are situated on the frontal surface at a distance from the sides, so that the cirri do not reach the rounded edges of the broad principal filaments.

Activity of latero-frontal cirri. As indicated in Table II the latero-frontal cirri showed great variability in the degree of activity and in orientation of inactive cirri, as observed on nonstimulated isolated gill fragments. The cirri on the gill fragments of *Crassostrea cucullata* were most regular in behavior, practically all beating steadily at an angle of about 90°. On the gill fragments of the other species examined, the activity of the cirri was less regular. Mostly, the cirri were at rest occupying various positions between horizontal and vertical to the surface of the gill. In *Tridacna maxima,* the activity pattern tended to change with time. During the day of preparation, most cirri were at rest in the horizontal position. On the third day, beating at a small angle close to the vertical position predominated. In *T. squamosa,* most cirri were also at rest on the first day, but occupying all positions between the horizontal and vertical. Gill fragments of more specimens of the various species must be examined before it can be safely concluded that the patterns described are typical for the species.

In *Mytilus edulis* it was previously found that addition of 5-HT to the ambient medium in concentration of about 10^{-5} M caused regularly beating latero-frontal cirri to discontinue beating and to remain in the vertical position (Jørgensen, 1975). This reaction was also observed in *Crassostrea cucullata*, in which most latero-frontal cirri ceased beating and remained motionless in the vertical position when 5-HT was added to provide a concentration in the medium of 10^{-5}–10^{-4} M. However, the response was less complete than in *M. edulis,* some cirri continuing to beat sporadically, though often at a reduced angle. In other species, the response of the latero-frontal cirri to stimulation with 5-HT was less pronounced or almost lacking. However, the type of response was generally similar to that observed in *M. edulis* and *C. cucullata,* that is, reduction of the angle of beat or stoppage in a position oriented obliquely or vertically to the surface of the gill. *Crassostrea lugubris* seems to represent an extreme with respect to the response of the small latero-frontal cirri toward stimulation with 5-HT. In two out of three fragments observed, the cirri were at rest on the unstimulated preparation, oriented in positions between horizontal and vertical. In these preparations, addition of 5-HT to a concentration of 10^{-5}–10^{-4} M reestablished extensive beating of the cirri at angles of 90°. Increasing the concentration of 5-HT about 10^{-4} M reduced the number of beating cirri, and most of those brought to rest remained in the vertical

TABLE III

Effect of 5-HT on creeping rate of gill fragments of Pycnodonte numisma. *Figures indicate mean values in µm/sec; ranges are in parentheses. N is the number of readings.*

Preparation number	Concentration of 5-HT					
	0	N	10^{-6} M	N	10^{-5} M	N
1	320 (290–350)	3	160 (140–170)	4	130 (125, 130)	2
2	260	1	170	1	140	1
3	320 (290–360)	5	190 (180–220)	5	130 (100–170)	9

position. More experiments are needed to clarify the reactions of the laterofrontal cirri to 5-HT in different species of bivalves.

Frontal cilia. The activity of the frontal cilia was not studied systematically in the present investigation. General conclusions concerning their activity can, however, be drawn from the observations recorded above on the behavior of particles moving along the gill surface. These observations indicated that most frontal ciliary tracts continue beating unaffected by the isolation of the gill fragment from the rest of the body. In species with plicate gills this statement especially applies to the frontal ciliary tracts of filaments within the interplical grooves. On the filaments constituting the crests, particle transport, and consequently activity of frontal ciliary tracts, was in some species slow or absent (Table II).

It was noticed that gill fragments of *Pycnodonte numisma*, which exhibited only a weak particle transporting current along the plical crests toward the ventral margin, when placed in sea water in a Petri dish, exhibited creeping behavior more consistently than gill preparations from most other of the species examined. The gill fragments included the ventral margin, which constituted the trailing edge of the creeping fragment. Presumably, the movements were caused by the beating frontal cilia of the plical crests. It is suggested that the mechanical stimulation of cilia caused by their contact with the bottom of the dish activated the cilia. This activation continued for at least 24 hours, the period during which the gill fragments were observed to maintain their creeping activity with undiminished vigor.

In some preparations the rate of creeping was measured and the effect of 5-HT studied. It can be seen from Table III that 5-HT reduced the rate and that the reduction was stronger at a concentration in the ambient medium of 10^{-5} M than at 10^{-6} M.

Giant cirri. Giant cirri have been described on gill filaments of many species of bivalves, especially on the frontal and abfrontal surfaces of the filaments (see Atkins, 1936, 1937a). They were also occasionally noticed in the present study, but in *Tridacna maxima* they were far more numerous than in any of the other species examined. Many giant cirri were present, especially along the ventral margin of the inner demibranch and on the frontal surface of the filaments. The cirri were oriented vertically on the surface and were inactive most of the time. 5-HT in concentrations of 10^{-5}–10^{-4} M had no effect on the cirri, which remained motionless.

Mucus

Removal of one valve and mantle in order to expose the gill or excision of gill fragments stimulated moderate secretion of mucus on the surface of the gill. In the absence of further stimulation the secretion of mucus soon decreased to low levels. Only in *Tridacna*, mucus tended to accumulate on the gill surface. In the other species examined the frontal ciliary tracts immediately carried the mucus to the ventral margin or the dorsal groove. The intensity of mucous secretion could, therefore, be estimated from the amounts of mucus that accumulated at the cut ends of the ciliary tracts of the ventral margin and dorsal groove. 5-HT did not affect mucous secretion.

The role played by mucus in the retention and transport of particles by the bivalve gill has been much debated. It is, therefore, of interest to note that only a minor part of *Tetraselmis* cells retained by the intact gills or gill fragments of the bivalves examined became entrapped in mucus and were carried directly on the ciliary collecting tracts. The majority of retained algal cells, and other particles, were carried in the water currents above the ciliary tracts. These concentrated suspensions of algae could be observed to leave the gill fragments at the cut ends of the ciliary tracts of ventral margins and dorsal grooves, to redisperse in the ambient medium. The redispersed algal cells did not tend to adhere to each other, and they continued swimming normally by means of their flagella, indicating that the cells had not become smeared with mucus during the process of retention and transport by the gill. Algae that did become entangled in mucus accumulated at the cut ends of the ciliary tracts, being unable to become resuspended in the water.

Discussion

Observations made on exposed or isolated gills or gill fragments from various types of bivalve gills show that such preparations continue to transport water, but they only inefficiently retain particles suspended in the water. This is contrary to gills in intact, undisturbed bivalves, which retain particles efficiently down to sizes of a few μm in diameter (*Mytilus edulis*, Jørgensen and Goldberg, 1953; Vahl, 1972a; Jørgensen, 1975; *Crassostrea virginica*, Jørgensen and Goldberg, 1953; Haven and Morales-Alamo, 1970; *Cardium edule*, Vahl, 1973a; *Chlamys opercularis, C. islandica*, Vahl, 1972b, 1973b).

Gill filaments of bivalves receive serotonergic innervation (Paparo, 1972; Paparo and Finch, 1972), which seems to be cilio-excitatory (Aiello, 1960, 1970; Gosselin, 1961). Exogenous 5-HT may stimulate the rate of water transport through gill preparations, but the drug did not restore retentiveness of the gill. If anything, the gill became more leaky to 10–20 μm *Tetraselmis* cells added to the ambient medium, probably an effect secondary to an increased rate of water flow through the interfilamentar spaces.

MacGinitie concluded from studies published already in 1941 that only undisturbed suspension feeding bivalves feed normally. His observations had, however, little impact on subsequent studies on the function of the gill in feeding bivalves. Investigators have continued to examine mechanisms of particle transport and sorting on preparations, including bivalves with the gills exposed by removal of one valve or part of a valve and mantle, or isolated gill preparations (*e.g.*, Nelson, 1960; Stasek, 1962; Galtsoff, 1964; Morton, 1969; Fankboner, 1971; Narchi, 1972; Bernard, 1974).

One reason for the continued interpretation of observations on how particles are dealt with by exposed gills or by gill fragments in terms of normal feeding mechanisms is probably to be found in the apparent expediency in the functioning of such preparations. They continue performing complex activities, such as straining, sorting, and transporting particles, as described in detail in numerous species of bivalves, especially by Atkins (1936, 1937a, b, 1938). Less emphasis was placed on the observation, in most cases casually mentioned (Galtsoff, 1964; Bernard, 1974), that removal of merely part of one valve and mantle caused leakiness of the gill. Such preparations may survive in the laboratory for several months and appear to function normally (*Crassostrea gigas,* Bernard, 1974). From observations on three species of oysters with one mantle removed and kept in sea water from a circulation tank for several months, Nelson (1960) concluded that undisturbed oysters in clean water transport water through the gills without retaining suspended particles. Only addition of sufficient amounts of suspensions of carmine or phytoplankton induced retention of particles by causing contractions of ostia and filaments.

Experiments of the type made by Nelson, Galtsoff and Bernard illustrate how resistant bivalves can be toward mutilation with respect to ability to survive. This hardiness of bivalves toward adverse conditions has concealed that bivalves are also highly sensitive toward handling and changes in their normal environment. Presumably, one of the functions most easily impaired is the normal feeding activity of the gills.

It remains to be understood how the bivalve gill transports water at high rates and efficiently retains small particles. It has been suggested that the activity of the latero-frontal cirri is responsible for efficient retention (Dral, 1967; Moore, 1971; Owen, 1974a, b). This hypothesis cannot apply to species that lack latero-frontal cirri, such as *Anomia, Pteria* and pectinids (Vahl, 1973b).

According to MacGinitie, undisturbed bivalves produce while feeding a continuous sheet of mucus covering the gill surface and acting as a filter that strains even colloidal particles from the passing water (MacGinitie, 1941, 1945). There is no direct evidence to support MacGinitie's mucous sheet hypothesis. It seems to be inconsistent with the finding in several species of bivalves that the critical particle size for complete retention is about 2–4 μm in diameter. The role played by mucus in normal feeding remains to be elucidated. Secretion of mucus in amounts that may form sheets on the gill surface seems to serve cleaning purposes (Bernard, 1974; see also Jørgensen, 1966, for discussion.)

The finding that even slight disturbance of bivalves produces leaky gills suggests great lability of the functional organization of the ciliary system in the undisturbed feeding bivalve.

5-HT was observed to reduce the creeping rate of gill fragments of *Pycnodonte numisma.* Creeping rates of gill fragments have often been used to measure activity of the frontal cilia. However, Gosselin and O'Hara (1961) found that the effects of 5-HT on the rate at which the gill surface (*e.g.,* in *Mytilus edulis*) transported particles depended upon the size of the particles. 5-HT enhanced the rate of transport of small particles and reduced the rate of large particles. The authors explain these paradoxical effects of 5-HT by assuming that the motion of large particles along the frontal surfaces of the filaments is retarded by the perpendicular current of water generated by the lateral cilia, and that this retardation becomes more evident when the lateral cilia are stimulated by 5-HT. Jørgensen

(1975) observed that in the 5-HT stimulated gill of *Mytilus edulis* the latero-frontal cirri occupy positions vertical on the gill surface with their tips curved over the frontal surface of the filaments. In the experiments with the gill fragments of *Pycnodonte,* 5-HT both stimulated the activity of the lateral cilia and tended to arrest the latero-frontal cirri in a vertical position. Both effects may have contributed to the reduced creeping rates.

In conclusion, three functional states can be distinguished in the gills of suspension-feeding bivalves.

First, the *nonretentive* state is characteristic of disturbed animals and gill preparations. This state seems to have predominated in experiments on measurements of water transport by means of direct methods (Jørgensen, 1966). The functional significance of the state is not clear.

Secondly, *the cleaning state* is characterized by copious mucous secretion from the gill surface and activity of gill musculature. The state is typically elicited by high concentrations of suspended matter in the surrounding water. It has often been studied on exposed gills, whose function has been examined by means of thick suspensions added on to the gill surface.

Thirdly, *the feeding state* is characterized by high rates of water transport through highly retentive gills. This functional condition, which is presumably the phylogenetically youngest, is restricted to undisturbed bivalves. In studies on the functions of the bivalve gill, the feeding state has, therefore, mainly been approached indirectly.

The mechanisms by which the bivalve gills transport water at high rates and retain particles efficiently are not well understood. They may involve a functional integration of the different ciliary tracts on the frontal and lateral surfaces of the filaments.

I extend my warm thanks to Professor Zeev Reiss, to Dr. Ilan Paperna, Director, and to the staff of the H. Steinitz Marine Biology Laboratory, Elat, Israel, for hospitality and generous help during my stay as a Gudelsky Fellow. Professor Georg Haas and Dr. Henk K. Mienis, Department of Zoology, The Hebrew University of Jerusalem, and Dr. Jørgen Knudsen, Zoological Museum, University of Copenhagen, identified the bivalves examined. I gratefully acknowledge their help in the difficult and laborious task of identification of the young specimens studied.

SUMMARY

1. Observations were made on muscular and ciliary activity and particle transport and retention in intact gills and gill fragments of the suspension-feeding, epifaunal bivalves *Anomia achaeus* Gray, *Juxtamusium maldivense* (E. A. Smith), *Pteria macroptera* (Lamarck), *Pycnodonte hyotis* (L.), *P. numisma* (Lamarck), *Crassostrea cucullata* (von Born), *Crassostrea lugubris* (Sowerby), *Tridacna maxima* (Röding), and *T. squamosa* Lamarck.

2. Gill contractions were especially violent in *Pteria* and both species of *Pycnodonte.* 5-HT in concentrations of 10^{-4}–10^{-5} M caused relaxation of con-

tracted gills, and reduced or abolished the response to mechanical stimulation, except in *Tridacna*.

3. In *Juxtamusium,* the concertina-like movements of the gill plicae could be correlated with the functional state of the water-transporting lateral cilia. When all lateral cilia were active, and the plicae inflated, movements of the plicae were discontinued. At periods of arrest of the lateral cilia the plicae collapsed and resumed concertina-like movements. 5-HT had no clear effect on the concertina-like movements of the gill plicae, except in *Juxtamusium* in which addition of 5-HT to the ambient medium stimulated the movements.

4. Exposed gills or gill fragments of all the species examined continued to transport water, but only inefficiently retained particles, 10–20 μm *Tetraselmis* cells, added to the water. 5-HT enhanced the rate of water transport in most species by stimulating the activity of the lateral cilia, but reduced the ability of the gill to retain *Tetraselmis* cells. The cilio-excitatory nerve transmitter 5-HT of bivalve gill filaments thus did not restore normal feeding activity of the gill.

5. Latero-frontal cirri could not be distinguished on the gill filaments of *Anomia, Juxtamusium,* and *Pteria.* In *Crassostrea lugubris* they were small (*ca.* 13 μm, in length) and inconspicuous. They were about 15 μm long in the two species of *Tridacna,* and about 17 μm long in *Crassostrea cucullata* and in the two species of *Pycnodonte,* spanning about half of the interfilamentar space in relaxed plicae. The latero-frontal cirri varied greatly in activity and in orientation of inactive cirri. Also the effects of 5-HT were variable, but the drug tended to arrest the cirri in an erect position.

6. 5-HT reduced the creeping rate of gill fragments of *Pycnodonte numisma.*

7. The gill fragments secreted mucus at low rates even in dense suspensions of *Tetraselmis* cells. The majority of *Tetraselmis* cells that were retained and transported by the gill fragments remained free of mucus, to be redispersed in the medium when they arrived at cut ends of the particle transporting ciliary tracts along the gill bases or the ventral margins of the demibranchs.

8. It is concluded that the feeding state of the bivalve gill, which is characterized by high rates of water transport through highly retentive gills, is restricted to undisturbed, intact animals. The mechanisms of feeding, therefore, cannot be finally understood from studies on exposed gills or gill fragments. The physiological significance of the nonretentive gill of disturbed animals and gill preparations is not clear.

LITERATURE CITED

AHMED, M., 1975. Speciation in living oysters. *Adv. Mar. Biol.,* **13**: 357–397.

AIELLO, E. L., 1960. Factors affecting ciliary activity on the gill of the mussel *Mytilus edulis. Physiol. Zool.,* **33**: 120–135.

AIELLO, E. L., 1970. Nervous and chemical stimulation of gill cilia in bivalve molluscs. *Physiol. Zool.,* **43**: 60–70.

AIELLO, E., AND M. A. SLEIGH, 1972. The metachronal wave of lateral cilia of *Mytilus edulis. J. Cell Biol.,* **54**: 493–506.

ATKINS, D., 1936. On the ciliary mechanisms and interrelationships of lamellibranchs. Part I. Some new observations on sorting mechanisms in certain lamellibranchs. *Quart. J. Microsc. Sci.,* **79**: 181–308.

ATKINS, D., 1937a. On the ciliary mechanisms and interrelationships of lamellibranchs. Part II. Sorting devices on the gill. *Quart. J. Microsc. Sci.,* **79**: 339–373.

ATKINS, D., 1937b. On the ciliary mechanisms and interrelationships of lamellibranchs. Part III. Types of lamellibranch gills and their food currents. *Quart. J. Microsc. Sci.*, **79**: 375–421.

ATKINS, D., 1938. On the ciliary mechanisms and interrelationships of lamellibranchs. Part VII. Latero-frontal cilia of the gill filaments and their phylogenetic value. *Quart. J. Microsc. Sci.*, **80**: 345–436.

BERNARD, F. R., 1974. Particle sorting and labial palp function in the Pacific oyster *Crassostrea gigas* (Thunberg, 1795). *Biol. Bull.*, **146**: 1–10.

DRAL, A. D. G., 1967. The movements of the latero-frontal cilia and the mechanism of particle retention in the mussel (*Mytilus edulis* L.). *Netherlands J. Sea Res.*, **3**: 391–422.

FANKBONER, P. V., 1971. The ciliary currents associated with feeding, digestion, and sediment removal in *Adula* (*Botula*) *falcata* Gould 1851. *Biol. Bull.*, **140**: 28–45.

GALTSOFF, P. S., 1964. The American oyster *Crassostrea virginica* Gmelin. *U. S. Fish Wildlife Serv. Fish. Bull.*, **64**: 1–480.

GOSSELIN, R. E., 1961. The cilio-excitatory activity of serotonin. *J. Cell. Comp. Physiol.*, **58**: 17–26.

GOSSELIN, R. E., AND G. O'HARA, 1961. An unsuspected source of error in studies of particle transport by lamellibranch gill cilia. *J. Cell. Comp. Physiol.*, **58**: 1–9.

HAVEN, D. S., AND R. MORALES-ALAMO, 1970. Filtration of particles from suspension by the American oyster *Crassostrea virginica*. *Biol. Bull.*, **139**: 248–264.

JØRGENSEN, C. B., 1966. *Biology of suspension feeding*. Pergamon Press, Oxford, 357 pp.

JØRGENSEN, C. B., 1975. On gill function in the mussel *Mytilus edulis* L. *Ophelia*, **13**: 187–232.

JØRGENSEN, C. B., AND E. D. GOLDBERG, 1953. Particle filtration in some ascidians and lamellibranchs. *Biol. Bull.*, **105**: 477–489.

KNIGHT-JONES, E. W., 1954. Relations between metachronism and the direction of ciliary beat in Metazoa. *Quart. J. Microsc. Sci.*, **95**: 503–521.

MACGINITIE, G. E., 1941. On the method of feeding of four pelecypods. *Biol. Bull.*, **80**: 18–25.

MACGINITIE, G. E., 1945. The size of the mesh openings in mucous feeding nets of marine animals. *Biol. Bull.*, **88**: 107–111.

MOORE, H. J., 1971. The structure of the latero-frontal cirri on the gills of certain lamellibranch molluscs and their role in suspension feeding. *Mar. Biol.*, **11**: 23–27.

MORTON, B., 1969. Studies of the biology of *Dreissena polymorpha* Pall. 1. General anatomy and morphology. *Proc. Malacol. Soc. London*, **38**: 301–321.

NARCHI, W., 1972. On the biology of *Iphigenia brasiliensis* Lamarck 1818 (Bivalvia, Donacidae). *Proc. Malacol. Soc. London*, **40**: 79–91.

NELSON, T. C., 1960. The feeding mechanism of the oyster II. On the gills and palps of *Ostrea edulis*, *Crassostrea virginica* and *C. angulata*. *J. Morphol.*, **107**: 163–203.

OWEN, G., 1974a. Feeding and digestion in the Bivalvia. *Advan. Comp. Physiol. Biochem.*, **5**: 1–35.

OWEN, G., 1974b. Studies on the gill of *Mytilus edulis:* the eulatero-frontal cirri. *Proc. Roy. Soc. London Ser. B.*, **187**: 83–91.

PAPARO, A., 1972. Innervation of the lateral cilia in the mussel *Mytilus edulis* L. *Biol. Bull.*, **143**: 592–604.

PAPARO, A., AND G. E. FINCH, 1972. Catecholamine localization, content, and metabolism in the gill of two lamellibranch molluscs. *Comp. Gen. Pharmacol.*, **3**: 303–309.

STASEK, C. R., 1962. The form, growth and evolution of the *Tridacnidae* (Giant Clams). *Arch. Zool. Exp. Gén.*, **101**: 1–40.

STEPHANO, G. B., AND E. AIELLO, 1975. Histofluorescent localization of serotonin and dopamine in the nervous system and gill of *Mytilus edulis* (Bivalvia). *Biol. Bull.*, **148**: 141–156.

VAHL, O., 1972a. Efficiency of particle retention in *Mytilus edulis* L. *Ophelia*, **10**: 17–25.

VAHL, O., 1972b. Particle retention and relation between water transport and oxygen uptake in *Chlamys opercularis* (L.) (Bivalvia). *Ophelia*, **10**: 67–74.

VAHL, O., 1973a. Porosity of the gill, oxygen consumption and pumping rate in *Cardium edule* (L.) (Bivalvia). *Ophelia*, **10**: 109–118.

VAHL, O., 1973b. Efficiency of particle retention in *Chlamys islandica* (O. F. Müller). *Astarte*, **6**: 21–25.

Reference *Biol. Bull.,* **151**: 344–356. (October, 1976)

FLOW AND FEEDING IN FAN-SHAPED COLONIES OF THE GORGONIAN CORAL, *LEPTOGORGIA*

GORDON J. LEVERSEE[1]

Department of Zoology, Duke University, Durham, North Carolina 27706

Many polypoid suspension feeders are fan-shaped and orient perpendicular to prevailing water currents (Riedl, 1966). Perpendicular orientation of gorgonians has been described as due to hydrodynamic forces (Theodor and Denizot, 1965; Wainwright and Dillon, 1969; Grigg, 1972) and as an adaptation for greater feeding efficiency (Laborel, 1960; Barham and Davies, 1968). It is likely that the mechanical properties of the skeleton and the morphology and orientation of colonies are well-adapted to both feeding and support as stated by Wainwright and Dillon (1969).

The objectives of this study are first, to determine whether morphology and orientation of the sea-whip *Leptogorgia virgulata* can be correlated with prevailing tidal currents; and secondly, to test the hypothesis, in laboratory studies, that fan-shaped colonies oriented perpendicular to flow (Fig. 1) will have a feeding advantage over colonies oriented parallel to flow. Feeding rates are measured by the number of *Artemia* nauplii caught per unit time. The importance of morphology and orientation to the feeding success of passive suspension feeders is examined in light of the data collected.

MATERIALS AND METHODS

Field studies

The morphologies and orientation to currents of two populations of *Leptogorgia* in the vicinity of the Duke University Marine Laboratory, Beaufort, North Carolina, were surveyed using S.C.U.B.A. gear.

Channel population. One population was found in a channel in 5–7 meters of water. The colonies were attached to shell and rubble covered by smooth sand. The dominant hydrodynamic forces in this protected area were due to daily bidirectional tidal currents. A 10 × 2 meter grid was laid out across the bottom of the channel and current speeds and direction were measured at the 0 m, 5 m, and 10 m points across the grid, 0.5 m off the bottom. Current speed was determined by timing the passage of suspended particles across a 1 m distance and was found to range from 0–0.25 m/sec (0–0.5 knots) depending on tidal stage. Current direction was recorded by measuring the compass bearings of fine nylon streamers. Neither current speed nor direction were found to vary significantly across the grid.

Morphology and orientation of colonies within the grid were determined. The degree of planar or fan-shaped morphology is indicated by the thickness to width ratios of 1 : 5, 1 : 4, 1 : 3, 1 : 2, 1 : 1 (Fig. 1). A ratio of 1 : 5 indicates a high degree of fan-shaped morphology, while a ratio of 1 : 1 indicates that the branches

---

[1] Present address: Biology Department, Nasson College, Springvale, Maine 04083.

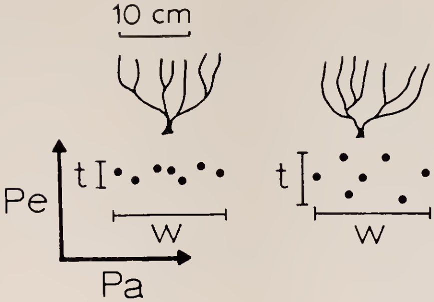


FIGURE 1. Whole fan-shaped colonies as seen upright (top) and as branch tips would appear when seen from above (bottom). Thickness to width ratios (t: w) were calculated as a measure of fan morphology. Orientation to flow is classed as perpendicular (Pe) when flow direction intercepts the fan blade at right angles and parallel (Pa) when flow direction is colinear with width of the fan blade.

are distributed in a bushy, three-dimensional array. Thickness to width ratios were measured from tracings of branch tip positions made on a clear plastic plate positioned above colonies during slack tide. Orientations of planar colonies were measured during slack tide by visual orientation of a protractor to the plane of the colony and are expressed in degrees relative to previously recorded tidal current direction. An orientation of 90° indicates that the fan is perpendicular to the tidal current. Orientations are grouped in 10° classes.

Jetty population. The second population of *Leptogorgia* was found scattered over the surface of a rock jetty at the south-west corner of Piver's Island. This population was attached to rough broken concrete in 1–5 meters of water and was subjected to currents from two converging tidal channels in addition to swell caused by frequent passing of trawlers and pleasure boats. Current speed and direction were monitored at eight locations within a 20 m^2 grid using the methods described. Suspended particles showed turbulent currents with frequently changing speed and direction across the grid and even around single colonies.

Feeding studies

Feeding mechanisms and feeding rates. Fan-shaped colonies of *Leptogorgia* 0.1–0.15 meters in height, were placed in a recirculating water tunnel of 60 liter capacity with a working area 0.3 meter long by 0.2 meter square (Fig. 3). The tunnel was powered by a 1/30 H.P. heavy duty laboratory stirrer connected to a solid state motor control. Current speed was determined by timing with a stopwatch the passage of suspended particles in the center of the working area over a distance of 0.3 meter. The mean of ten determinations was used as the recorded speed, and the results of a typical determination are 0.04 ± 0.005 m/sec. The rpm of the motor were checked each hour during an experiment using a Model 1531 Strobotac (General Radio Co., Concord, Mass.) and were found to be constant ±3%. Plexiglass baffles and a laminator of plastic soda straws (individual straw, 0.25 × 8.25 inch, Sweetheart Straws, Maryland Cup Corp.) maintained a reasonably laminar flow. Except in the boundary layer 2 cm

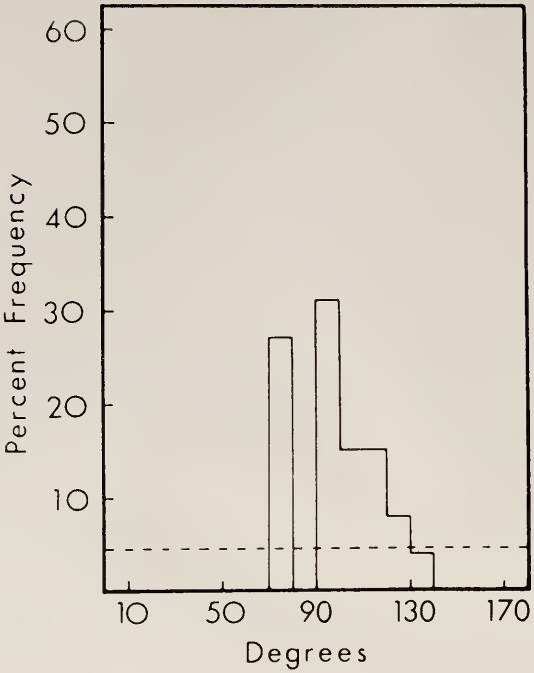


FIGURE 2. Orientation of fan-shaped colonies in the channel population relative to tidal current direction. Colonies are grouped in 10° classes, 90° representing an orientation perpendicular to the current. The probability that the observed distribution is a chance sampling from a random distribution (represented by dotted line) is less than 0.001 ($\chi_{25}^{2} = 299$).

thick, working area current velocities were equal to central velocity minus 1–10%. Current velocities in the boundary layer were found to be reduced by up to 50% below central velocity.

The tunnel was filled with sea water filtered through a 5 micron filter bag. Selected fan-shaped colonies were collected and held in filtered sea water for a 24-hour fasting and acclimation period before use. They were then placed in the tunnel oriented either parallel or perpendicular to the current direction and allowed to acclimate further for several hours before *Artemia* nauplii were added. Living *Artemia* nauplii were harvested from culture 24 hours after eggs had been

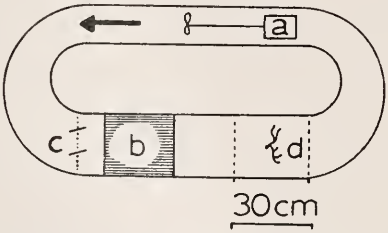


FIGURE 3. Recirculating water tunnel seen from above: lab stirrer (a), laminator (b), baffles (c), and working area with colony (d). Flow direction is indicated by arrow.

added to filtered sea water. *Artemia* carapace length was 0.48 ± 0.03 mm, $n = 20$. The *Artemia* nauplii were counted by eye using a 10 ml pipet and added to the tunnel until a final concentration of 20 ± 3 per liter was obtained. The number of *Artemia* in the tunnel was determined directly by taking 4–6 one liter samples using a 1000 ml beaker. Samples were pourer through a 47 mm Millipore filter apparatus under gentle aspiration; the cellulose filters were replaced with a square of #20 mesh nylon plankton netting. The *Artemia* nauplii were counted by eye on the netting and returned to the tunnel along with the filtered sea water. Tests showed that *Artemia* so handled survived as well as controls over a 24 hour period. During an experiment, *Artemia* nauplii were sampled every one to two hours over a four to six period.

At the end of each experiment, the colony was removed and held in filtered sea water for 24 hours. It was then returned to the tunnel, which had been drained and refilled, rotated 90° from its orientation in the first experiment. Some colonies were tested first in a perpendicular orientation followed by parallel orientation, and other colonies were tested in reverse order. Altogether twelve orientation-feeding experiments on six different colonies were completed.

Calculation of feeding rates. Feeding rates in this study are expressed as percentage of *Artemia* consumed per colony per unit time rather than the more conventional "clearance rate" for the reasons discussed below.

Active suspension feeders can be defined as those which maintain a feeding current using cilia or appendages. Jørgensen (1949) has shown that in a closed suspension-feeding system, the concentration of food particles will decrease exponentially with time according to the following formula: $\text{conc}_t = \text{conc}_0 \times e^{-(mt/M)}$, where conc_t represents the concentration of particulate food in the system at time t; conc_0 the concentration of food at time 0; M the volume of the tank; m the volume of water "cleared" of food at time t; and e the base of natural logarithms. Calculation of the clearance rate, the volume of water cleared per unit time, is frequently used as an expression of feeding rate in active suspension feeders, and depends on the volume of water pumped and particle retention of catch efficiency (number of particles retained number of particles encountered). For active suspension feeders, the container can be considered a well-stirred volume, and particle retention will not vary with ambient currents unless current velocities are so high or so low as to interfere with the feeding current generated by the animal or with the distribution of food in the system.

For a passive suspension feeder like *Leptogorgia*, feeding on large particles, ambient currents are analogous to feeding currents or the volume of water pumped by active suspension feeders. As ambient current velocity ("pumping rate") increases, the clearance rates will *increase*. It is apparent from observations, however, that at high current velocity (≥ 0.5 m/sec) bending of the colony and polyps reduces the likelihood of *Artemia* capture (*i.e.*, above a certain current velocity, particle retention or catch efficiency decreases, with a resulting *decrease* in clearance rate). These counteracting effects of increasing current velocity bring into question the use of clearance rates as a measure of feeding in passive suspension feeders, and point out the need to carefully specify the current regime under which experiments are conducted. The use of colonies in different orientations at similar current speeds and with similar initial *Artemia* concentrations allows the use of

Table I

Thickness to width ratios of colonies of Leptogorgia *from two locations. The probability that the frequency of fan-shaped colonies is the same in both locations is less than 0.001* ($x_4^2 = 33.3$).

Fan-shaped morphology	Thickness to width ratio	Number of colonies	
		Channel	Jetty
Excellent	1.5	11	2
Good	1.4	13	4
Fair	1.3	3	3
Poor	1.2	7	6
None	1.1	2	27
		36	42

the simpler percentage of *Artemia* consumed/time as a measure of feeding rate. Feeding rates were calculated from best fit plots of log percentage of *Artemia*/liter *vs.* time in hours, over a period of four hours.

The current velocity chosen (0.04 m/sec) lay within the range of currents measured in field studies and did not cause severe bending of the colonies or polyps. The *Artemia* concentration of 20/liter was high enough to measure reliably using the sampling technique described, but low enough to avoid satiation of the colonies. The estimated number of polyps per colony ranged from 5–20 × 10^3, with the total number of *Artemia* in the tunnel at about 1–1.5 × 10^3. The *Artemia* concentration of 20/liter is 10–20 times higher than the natural zooplankton population in the Beaufort area (W. Kirby-Smith, Duke University, personal communication), but is closer to natural conditions than the 2–3 × 10^3/liter used in previous suspension-feeding experiments (Crisp and Southward, 1961)

Results

Field studies

The data in Table I indicate that there is a much higher incidence of colonies with a low thickness to width ratio in the channel population, subjected to bi-directional currents, than there is in the jetty population, subjected to unpredictable turbulent currents. This result is similar to that of Grigg (1972) who found that colonies of *Muricea* assumed more fan-like morphologies in the presence of strong bi-directional currents.

The fan-shaped colonies of the channel population have a strong preferred orientation perpendicular to the prevailing tidal currents (Fig. 2) Even if the resting orientation deviates some 20–30°, the comparatively spindly *Leptogorgia* is easily twisted into a 90° orientation by currents. When tidal currents were running, fans invariably were oriented perpendicular to current direction indicated by the path of suspended particles. These data support subjective impressions of divers who reported seeing populations of *Leptogorgia* with fan-shaped morphologies and uniform orientation to current direction (R. Searles, Duke University, personal communication). *Leptogorgia,* then, is another example of a gorgonian coral of variable morphology showing a high incidence of fan-shaped colonies oriented perpendicular to directional currents and can be used to test the hypothesis that this orientation confers some feeding advantage.

Feeding studies

Feeding rates of each colony in perpendicular and parallel orientation were calculated from best fit linear regression plots of log # *Artemia*/liter *vs.* time in hours. The semi-log plot (Fig. 4) is an estimate of feeding rate (Jørgensen, 1949). Observed differences in slopes for parallel and perpendicular orientation were tested for significance by analysis of covariance (Snedecor and Cochran, 1967, p. 433). For ease of presentation, feeding rates are presented in Table II as the percentage of *Artemia* consumed/colony/hour.

It can be seen in Table II that in all cases, colonies in a perpendicular orientation capture as many or more *Artemia* per unit time than the same colonies oriented parallel to flow. The range of differences is great and not all differences are

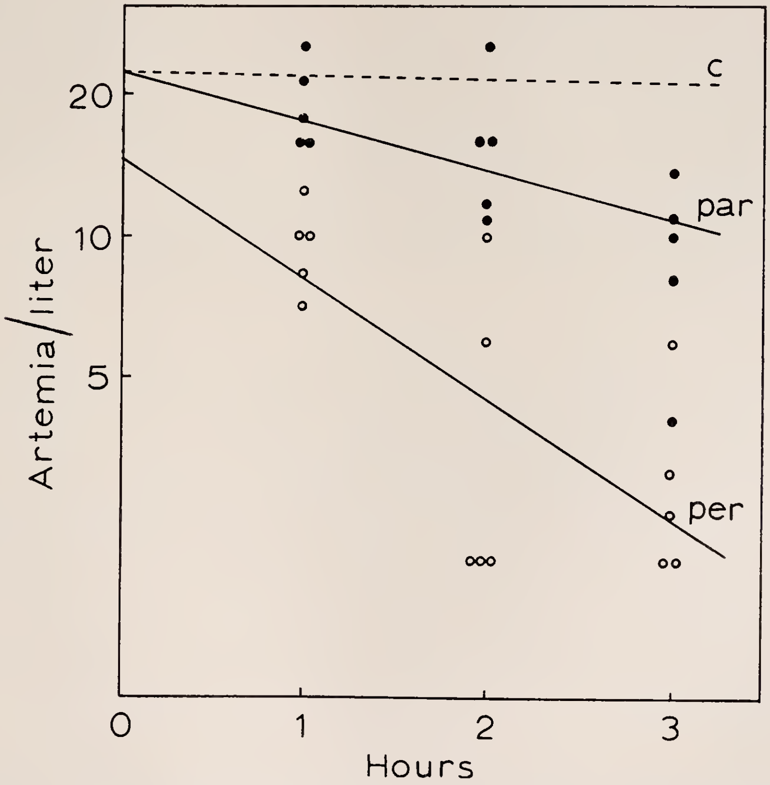


FIGURE 4. Best fit linear regression of log # *Artemia*/liter vs. time for colony #1 water tunnel feeding experiments. Slopes are a measure of feeding rate and are significantly different ($F_{1,36} = 6.36$, $P < 0.05$) for perpendicular orientation (open circles, $y = -0.2594x + 1.6990$) *vs.* parallel orientation (closed circles, $y = -0.1184x + 1.3670$). Experiments in which colonies did not expand are used as controls (C, dashed line) and show no significant loss of *Artemia* over 18 hours.

TABLE II

Feeding rates of fan-shaped colonies in parallel vs. *perpendicular orientation relative to tunnel water current. Feeding rates were calculated from best fit linear regression of log #* Artemia/*liter* vs. *time in hours, and are expressed as %* Artemia *consumed/hour. The significance of feeding rate difference for each colony as determined by analysis of covariance (Snedecor and Cochran, 1967, p. 432) is listed in parenthesis (n.s. = not significant). Experiments are listed in chronological order.*

Colony	Orientation	Feeding rate % *Artemia*/hr	Rate difference perpendicular-parallel
1	Perpendicular	28.5	+ 5.5 (n.s.)
	Parallel	23.0	
2	Perpendicular	39.6	+ 15.6 ($P < 0.05$)
	Parallel	24.0	
3	Parallel	24.5	+ 52.2 ($P < 0.05$)
	Perpendicular	76.7	
4	Parallel	34.5	+ 6.5 (n.s.)
	Perpendicular	41.0	
5	Parallel	38.6	+ 7.0 ($P < 0.05$)
	Perpendicular	45.6	
6	Perpendicular	66.2	+ 43.9 ($P < 0.01$)
	Parallel	22.3	

significant for single experiments, but the trend is clear enough to suggest that the phenomenon is real. For the first time, experimental evidence shows that fan-shaped colonies do have a feeding advantage when oriented perpendicular to flow, regardless of the actual mechanisms which control morphology and orientation. The question of feeding as related to colony morphology is explored in the following discussion.

DISCUSSION

Colonies of *Leptogorgia* in the Beaufort area are typically 0.2–0.3 meters in height, with a maximum height of probably 0.5 meters. The colonies used in feeding studies were 0.1–0.15 meters in height and fan-shaped, *i.e.*, no branch was located downstream of another branch. The polyps on colonies of this size average about 1 mm in length by 0.5 mm in width, with a spread of expanded tentacles of about 1 mm diameter, although these measurements vary greatly with degree of expansion of individual polyps. The spicular coenenchyme axis of each branch when expanded may be 0.5–2.0 mm in diameter, with polyps more or less randomly distributed around the circumference and along the axis of each branch. Some other gorgonian corals have very regular arrangements of polyps, and careful studies of *Leptogorgia* may reveal subtle patterns of polyp distribution which may affect feeding success.

Feeding rate measurements must take into account the willingness of the animal to feed. The assumption that corals with polyps expanded are feeding is not necessarily valid. When an *Artemia* encounters an actively feeding polyp, it will be enveloped by all tentacles within 1–2 seconds and be conveyed to the mouth. An *Artemia* can be ingested within 5 seconds. Ingested specimens of *Artemia* are clearly visible in the gut cavity of individual polyps which have recently fed and can pass from the polyp out of view into the colonial gastrovascular cavity as rapidly as 1–2 minutes. As many as 5 feedings in 30 minutes by one polyp have been observed. The rate of passage of *Artemia* nauplii into the colonial gastrovascular cavity slows with repeated feeding.

There is a great range of individual polyp responses. Some polyps feed repeatedly, some polyps feed once and remain with tentacles contracted, some polyps evidently do not feed at all. Even with polyps fully expanded, it was apparent that a colony may stop feeding. An *Artemia* encounter with a nonfeeding polyp triggers an initial, often incomplete, tentacle response, but ends with polyp tentacles unfolding and the *Artemia* swimming off apparently unharmed. Feeding experiments using branch tips in test tubes show that feeding may stop with as few as 10% of the polyps having fed. Control of the feeding response in *Leptogorgia* and other corals is not understood.

Logic suggests that feeding in passive suspension feeders should be related to current direction and velocity, prey density, size, etc. In these studies, some preliminary evidence was obtained suggesting that colony feeding response is related to changes in current velocity, a 4–6 hour period approximating one tidal period, and changes in prey density.

Observations in the feeding experiments conducted suggest that contracted colonies quickly expand (to feed) when subjected to a current, and that colonies

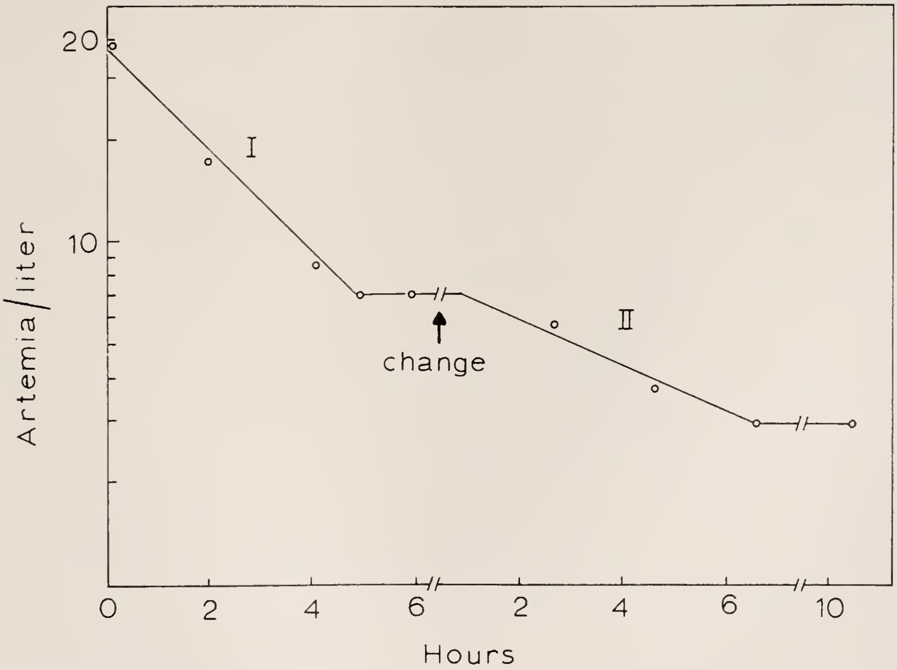


FIGURE 5. Cessation of feeding in two colonies after a 4–6 hour period approximating one tidal flow, regardless of prey density. Colony I fed at an initial *Artemia* concentration of 20/liter, stopped feeding after about 4 hours. Colony II fed for 4–6 hours when placed in the same water at the lower *Artemia* concentration (9/liter). This suggests that feeding may be linked to a 4–6 hour period rather than *Artemia* density. This type of result was found in two separate experiments using four different colonies of approximately the same size.

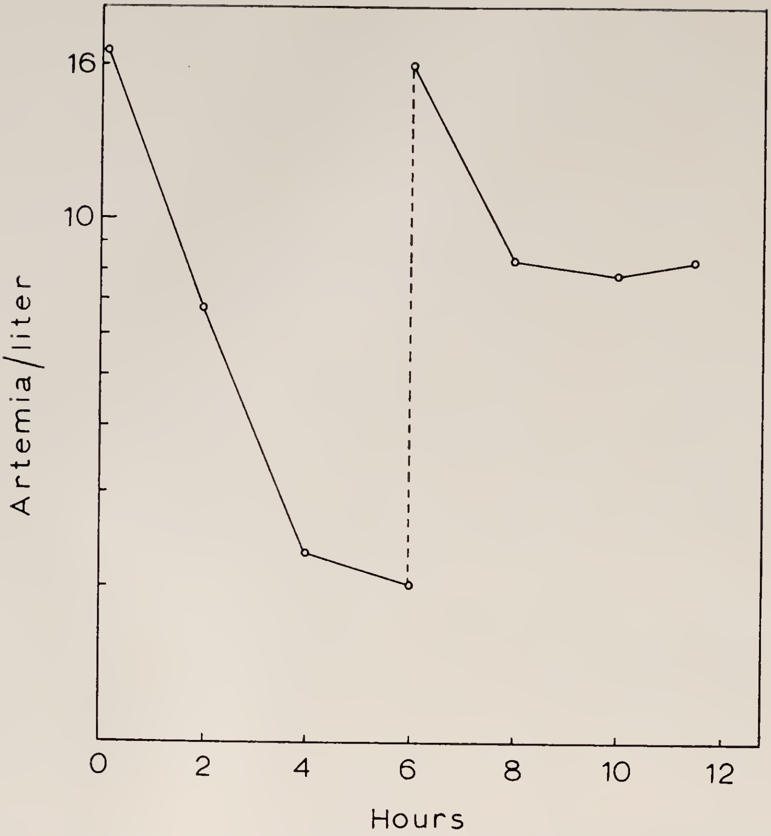


FIGURE 6. Induction of a colony feeding response by increasing *Artemia* concentration to initial levels (16/liter) after cessation of feeding at 4–6 hours. Similar results from two separate colonies suggest, that changing prey density is a factor in determining feeding response.

which contract after a period of exposure to a current tend to expand if current velocity is increased or decreased slightly. This sensitivity to change of current velocity was not tested systematically, but such sensitivity would be a likely mechanism for control of feeding response.

In the feeding experiments conducted, it was also noted that there was a tendency for colonies to stop feeding at 4–6 hours after "time 0" with final *Artemia* concentrations of from 2–5/liter. A single colony fed on four separate occasions at initial *Artemia* concentrations ranging from 20–60/liter, stopped feeding after 4–6 hours despite a range of final concentrations of from 2–25 *Artemia*/liter. This evidence suggests that cessation of feeding response is related to a 4–6 hour period following initiation of current flow and not to final prey density. Further evidence for this conclusion is found in results of two separate experiments which show that after one colony completes its 4–6 hour feeding period, a second colony will feed for 4–6 hours on the remaining prey (Fig. 5).

Experiments on two separate colonies suggest that changes in prey density can stimulate a colony feeding response. In these cases, addition of more *Artemia* at the end of the 4–6 hour feeding cessation induced a new but brief (two hour) feeding response (Fig. 6). This inducible feeding response would be adaptive in feeding on plankton "patches."

It is logical to assume that tidal periodicity, changes in current velocity, or changes in prey density might affect the responses of a passive suspension feeder. It is not clear, however, that any metabolic cost or effort is required for corals to remain constantly in a feeding state. Therefore, it is not clear what advantage is inherent in a periodic nonfeeding state. Perhaps these periodic feeding states, if real phenomena, are related to biochemical processes following a successful feeding period. Clearly, confirmation of these suggested feeding rhythms is in order before speculating further on their possible causes. Additional work in this area must avoid possible satiation effects which might affect feeding periodicity, and could involve careful determinations of prey/polyp ratios for fully fed colonies. These must be determined more carefully than my test tube determined 10–15%, and have their fasting conditions more carefully defined than the arbitrary 24-hour period used in this study.

The distribution of polyps around a branch of *Leptogorgia* seen in cross section is more or less uniform. One would expect only the upstream or side polyps to capture *Artemia*. In fact, downstream polyps catch many *Artemia* nauplii held in eddy currents in the downstream sides of the branches (Fig. 7). *Artemia* naupli may remain in these eddy currents for 15–20 seconds (personal observation). The *Artemia* nauplii swim or are carried by currents up and down the backside of branches and are often caught by feeding polyps. At current speeds in excess of about 0.04 m/sec the side polyps are swept back where they too feed in this eddy current. The generation of eddy currents by a colony may be a significant factor in feeding success.

Although fan-shaped colonies feed more rapidly with their broad surface facing the prevailing currents, the eddy currents noted may contribute to the feeding success of colonies which do not have a planar morphology. In a bushy, three

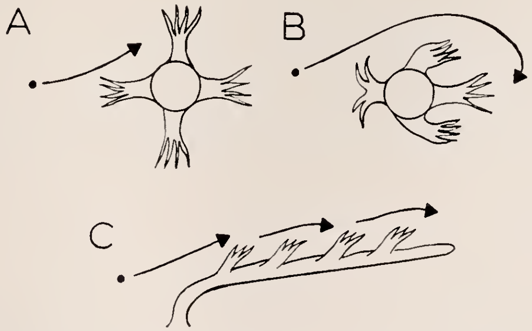


FIGURE 7. The effect of currents on movement of food particles (dot) and polyp position. In A (branch cross section), at current speeds of 0–0.05 m/sec, front and side polyps are at right angles to the branch and catch most prey. In B, at higher current speeds, side polyps tend to be bent back where they feed in eddy currents with rear polyps. In C, at current speeds ≥0.1 m/sec, branches bend over and prey have been seen to bounce from polyp to polyp until caught.

dimensional array of branches, the downstream branches would be feeding in the eddy currents of upstream branches. The colony would thereby have several opportunities to catch a single *Artemia*, compared to the single line of branches in a planar colony. This three dimensional array may catch a very high percentage of the *Artemia* which pass through it, and it remains to be seen whether the greater cross-sectional surface area of a fan-shaped array is actually the most advantageous as far as feeding is concerned.

At higher current speeds (≥ 0.1 m/sec), there is a tendency for colonies to be bent over, and for the polyps to be pressed against the branch surface. The reduced colony area presented to the current and the disadvantageous bending of the polyps would be expected to reduce feeding considerably. This is very likely the case, but *Artemia* nauplii encountering a feeding polyp can be swept away after a brief contact, only to be caught in the gauntlet of polyps downstream (Fig. 7). It is apparent, therefore, that feeding success and feeding "strategy" may change for both planar and bushy colonies at different current speeds, and quantitative data to this effect would be of interest.

In discussing the biological basis and probable selective advantage of a particular morphology and orientation in gorgonian corals, it is important to separate branching pattern effects from effects due to skewed growth patterns. This is especially true when discussing growth mechanisms since the same mechanisms may not be operating in each case.

In general, colony morphology is a result of branching pattern. The distinctly planar morphology of true sea fans, such as the genus *Gorgonia*, is clearly due to the anastomosing pattern of branching in a single plane. This pattern is so consistent as to suggest a close genetic control, such as the genetic determination of alternate *vs.* opposite branching in woody plants. Most gorgonians, however, have a more variable branching pattern and therefore more variable morphology. The planar morphology and orientation of the colony overall is determined as each branch is formed. Grigg (1972) proposes that hydrodynamic forces may be important in determining planar morphology in *Muricea*, but fails to relate these forces to branching pattern. He discusses instead how hydrodynamic forces may affect skewed growth, or warping of branches into a plane.

In fact, there is no data which relates to branching pattern in gorgonians. The high correlation between the presence of directional water currents and the occurrence of planar morphology still does not determine the cause of planar growth form. The processes which stimulate branching at the cellular and organismic level must be understood before the effects of extraorganismic factors can be determined.

Skewed growth due to differential skeletal synthesis is a factor which may affect colony morphology and certainly affects orientation of sea fans.

Wainwright and Dillon (1969) showed that in the genus *Gorgonia* taller sea fans tended to be oriented perpendicular to the prevailing water currents. Smaller colonies were more randomly oriented, reflecting the greater randomness of currents closer to the irregular reef surface. The authors concluded that hydrodynamic forces of prevailing currents twist the randomly oriented fan blades toward a perpendicular orientation such that forces across the fan blade will be equally distributed. The subsequent change in orientation occurs slowly as the fan grows taller and is fixed by differential skeletal synthesis.

Gorgonians with more variable morphologies also seem to exhibit differential skeletal synthesis. As previously noted, the planar morphology and perpendicular orientation to water currents of most gorgonians is primarily due to branching in a single plane. However, some branches arising out of this plane, may show signs of corrective growth or bending. This author has noted this in *Leptogorgia* and Grigg (1972) reported corrective growth in *Muricea*. In gorgonians, it seems that differential skeletal synthesis due to applied physical forces is a mechanism by which an existing skeletal component can be modified in shape or orientation. This mechanism has been reported previously in other skeletal systems, such as bone (Becker, Bassett, and Bachman, 1964) and wood (Kennedy and Farrar, 1965). But this mechanism is distinct from the effect of hydrodynamic forces on branching pattern, a poorly understood phenomenon at best.

This work demonstrates quantitatively that fan-shaped corals oriented perpendicular to water currents have a feeding advantage over fan-shaped colonies oriented parallel to currents. Given these results, it can be stated that this frequently observed fan-shape and orientation is an adaptation for feeding as well as for accommodation to hydrodynamic forces. The degree of bending of colonies at various current speeds may be adapted to maximize feeding while minimizing hydrodynamic drag. Experimental or theoretical investigations into the mechanical properties of the skeletal system as related to feeding and support would be of interest.

If one accepts the importance of colony morphology as a feeding adaptation, comparative studies on the distribution of species or morphological types according to current regime and food type should be encouraged. Evolutionary trends in morphological specialization or generalization as related to feeding in different current regimes can be expected. Most of all, the functional morphology of corals must be seen as a morphology related to flow, and thoughts about corals must be organized in a framework of flowing water.

My special thanks to Steve Wainwright and his research group LIMP, for stimulating my initial interest in corals, and for continued interest, support, and suggestions. My thanks to Bill Bretz for his water tunnel design and his shared ideas and expertise in flows and feeding. Thanks also to John Costlow and the people at DUML for their interest and assistance and to Marcie for putting up with it all. This work was supported in part by a National Science Foundation Summer Postdoctoral Award.

SUMMARY

Field studies demonstrate that the gorgonian coral *Leptogorgia virgulata* assumes a fan-shaped morphology oriented at right angles to prevailing tidal currents. Laboratory studies using a recirculating water tunnel and *Artemia salina* nauplii as food show that fan-shaped colonies oriented perpendicular to water currents capture more *Artemia* per unit time than the same colonies oriented parallel to water currents. Several feeding strategies which may operate at various current speeds are suggested. Possible mechanisms controlling feeding response and

the selective advantage of colony morphology and orientation as related to feeding and resistance to hydrodynamic forces are discussed.

LITERATURE CITED

Barham, E., and I. E. Davies, 1968. Gorgonian and water motion studies in the Gulf of California. *Underwater Naturalist,* **5**: 24–28.

Becker, R. O., C. A. Bassett and C. H. Bachman, 1964. Bio-electric factors controlling bone structure. Pages 209–232 in H. M. Frost, Ed., *Bone biodynamics.* Little, Brown and Co., Boston.

Crisp, D. J., and A. J. Southward, 1961. Different types of cirral activity of barnacles. *Phil. Trans. Roy. Soc. London Ser. B,* **243**: 271–307.

Grigg, R. W., 1972. Orientation and growth of sea fans. *Limnol. Oceanogr.,* **17**: 185–192.

Jørgensen, C. B., 1949. The rate of feeding by *Mytilus* in different kinds of suspension. *J. Mar. Biol. Ass. U.K.,* **28**: 333–344.

Kennedy, R. W., and J. L. Farrar, 1965. Tracheid development in tilted seedlings. Pages 419–453 in W. A. Coté, Ed., *Cellular ultrastructure of woody plants.* Syracuse University Press, Syracuse, New York.

Laborel, J., 1960. Contribution a'l'etude directe des peuplements benthiques sciaphiles sur substrat rocheux en Méditerranee. *Rec. Trav. Sta. Mar. Endoume,* **33**: 20–30.

Reidl, R., 1966. *Biologie des Meeresholen.* Verlag Paul Parey, Hamburg.

Snedecor, J., and W. G. Cochran, 1967. *Statistical methods,* 6th edition. The Iowa State University Press, Ames.

Theodor, J., and M. Denizot, 1965. Contribution a l'e'tude des gorgones, I. *Vie Mileu,* **16**: 237–241.

Wainwright, S. A., and J. R. Dillon, 1969. On the orientation of sea fans (Genus *Gorgonia*). *Biol. Bull.,* **136**: 130–139.

Reference *Biol. Bull.*, **151**: 357–369. (October, 1976)

HISTOCHEMICAL CHANGES IN GONADAL NUTRIENT RESERVES CORRELATED WITH NUTRITION IN THE SEA STARS, *PISASTER OCHRACEUS* AND *PATIRIA MINIATA*[1]

SISTER M. AQUINAS NIMITZ, O.P.

Department of Biology, Dominican College, San Rafael, California 94901

The annual reproductive cycle of a population of *Pisaster ochraceus* near Monterey, California, for the years 1954 through 1958 has been reported by Feder (1956), Greenfield (1959), and Farmanfarmaian, Giese, Boolootian, and Bennett (1958). Mauzey (1966) has reported the cycle for *Pisaster* at San Juan Island off the coast of Washington for 1962 and 1963. Comparable information is available for *Patiria miniata* for 1955 (Farmanfarmaian *et al.*, 1958) and from mid-1962 through early 1964 (Lawrence, 1965).

In addition to general delineation of the reproductive cycles for these two species, interest has been focused on biochemical changes occurring in rhythm with the cycles. Greenfield, Giese, Farmanfarmaian, and Boolootian (1958) have reported on lipid, protein, nonprotein nitrogen, and glycogen in the gonads and other organs of *Pisaster*, while Giese (1966a, b) gives more detailed information for both *Pisaster* and *Patiria*. Allen and Giese (1966) have investigated lipogenic rates in the gonads of *Pisaster*. Nimitz (1971) has reported histochemically detectable changes in the nutrient reserves of the gut of *Pisaster* and *Patiria*, as correlated with the reproduction and nutrition.

The present paper describes the localization of certain classes of fats and carbohydrates in the gonads of *Pisaster ochraceus* and *Patiria miniata* during the course of the reproductive cycle and correlates information gained through these histochemical studies with biochemical findings.

MATERIALS AND METHODS

Nimitz (1971) describes in detail the sources and processing procedures for the specimens of *Pisaster ochraceus* and *Patiria miniata* on which the studies reported here are based. Samples of gonads taken from two males and two females each month for two years were fixed, sectioned and stained with techniques for various classes of carbohydrate and lipid and occasionally for protein.

The relative abundance of oocytes in different stages of development at different times of year (Fig. 1) was determined by classifying 50 oocytes per specimen into one of the three size classes. The relative abundance of spermatocytes was determined by measuring the height of the columns of spermatocytes extending into the lumen. Sperm were noted as absent or present in small, moderate or large quantities.

RESULTS

Cyclic changes in the gonads

Pisaster and *Patiria* have two gonads in each arm and each empties separately by a short gonoduct at a pore in the angle between the arms. Both ovaries and

---

[1] Supported by USPHS grant GM-12312 to Dominican College.

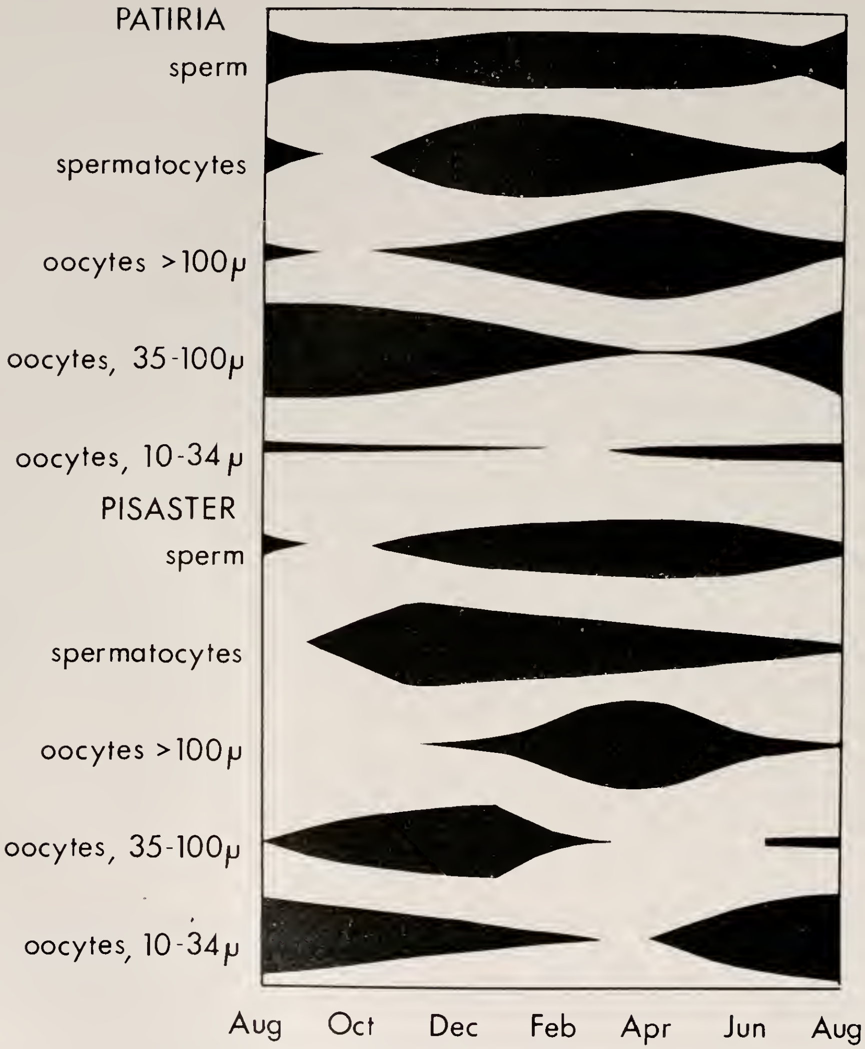


FIGURE 1. Seasonal abundance of germinal cells and sperm in the gonads of *Pisaster ochraceus* and *Patiria miniata*. The principle spawning period is indicated by the decrease in number of oocytes greater than 100 μ in diameter.

testes vary markedly in size in the course of the reproductive cycle in *Pisaster*. The gonads of *Pisaster* are smallest in August and September (GI = < 1), begin to enlarge about October, and reach a maximum size (GI = 16 or more) in March, April or May. Spawning occurred in May 1965 and between March and June 1966 (Nimitz, 1971, Fig. 7). Early in gametogenesis the testis and ovary are indistinguishable unless examined microscopically. Ripe ovaries are salmon pink; ripe testes, a cream color.

The gonadal cycle for *Patiria* is less pronounced and also less consistent. The gonads begin to increase in size in early fall. Over the period studied spawning occurred in July 1965 and in May of both 1966 and 1967 (Nimitz, 1971, Fig. 8). The lesser degree of synchronization of gametogenesis and spawning in *Patiria* is reflected in the smaller overall range within which the average monthly gonad indices fall (1.5 to 9.6) as compared with the range in *Pisaster* (0.5 to 19.7). Individual specimens of *Patiria* reach gonad indices ranging from 0.5 to 15.4, but these extremes do not show up in the monthly averages because of the presence of animals in any given sample tending to opposite extremes. The ripe ovaries of *Patiria* are a brownish-orange; the ripe testes, a cream color.

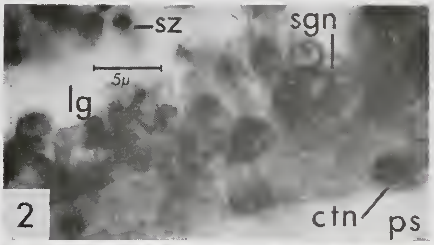
The histology and histochemistry of the gonads of freshly-collected specimens

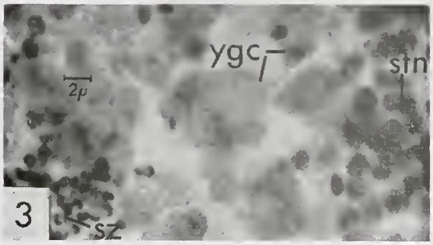
The following layers of the gonadal wall, distinguished in the combined light and electron microscopy studies by Davis (1971) and by Walker (1974), were likewise distinguished in the present study: the cuboidal peritoneal cells, the connective tissue layer, and the outer layer of muscle fibers of the outer sac; the perihaemal sinus (genital coelomic sinus); and the inner layer of muscle fibers, the haemal sinus and its walls, and the germinal epithelium of the inner sac. Other layers seen by electron microscopists could not be distinguished by the present investigator.

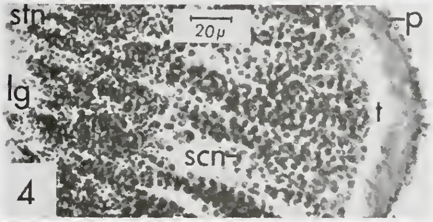
In a fully ripe testis from *Pisaster* with sperm filling the lumen (Fig. 2), there are along the connective tissue layer of the inner sac numerous oval cells 7 to 8 μ in length. The round to oval granular nucleus of each cell is 5 to 6 μ in diameter or length and contains a prominent nucleolus. These cells are interpreted as spermatogonia on the basis of the earlier work of Delavault and Bruslé (1968) on *Asterina gibbosa*. The lumen of the ripe testis is filled with sperm with spherical heads approximately 1.4 μ in diameter. At this stage, and in the succeeding one after the sperm have been released, there are often seen irregular yellowish granules in the peritoneal cells.

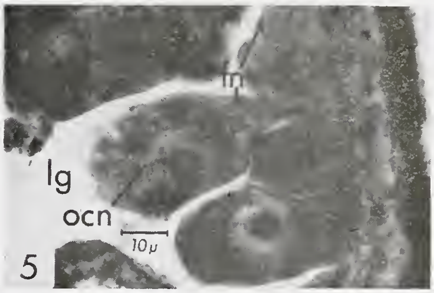
Ripe testes and recently-spawned testes also contain somewhat irregularly shaped cells approximately 10 μ in diameter with oval nuclei (2×3 μ) with a few basophilic granules. The cytoplasm of these cells is filled with yellow granules or globules, 0.5 μ in diameter (Fig. 3). These granules or globules stain with Sudan black B in both frozen and paraffin sections but were negative to all other stains utilized. Evidence which would permit identification of these cells either with the interstitial cells of *Asterina* testis (Delavault and Bruslé, 1968) or nutritive phagocytes of sea urchins (Holland and Giese, 1965) is lacking, though it seems unlikely that they are the same. They are distinctly different from cells of the vesicular tissue of *Asterina* in predominantly ovogenetic activity (Bruslé Tereygeol and Delavault, 1970).

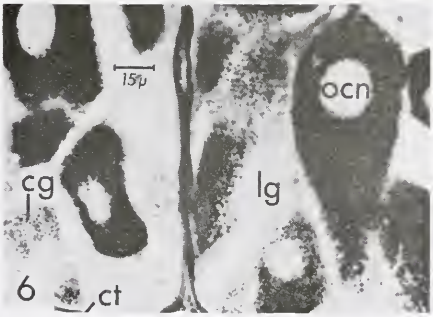
In a recently spawned testis the spermatogonia and other smaller cells are crowded together in irregular clusters along the edge of the testis because of the contraction of the wall after the release of the mass of sperm. As the spermatogonia divide, columns of cells presumed to be primary spermatocytes extend into the lumen of the testis (Fig. 4). The nuclei of the primary spermatocytes are approximately 2.5 μ in diameter and stain rather darkly, although a faint granulation can be distinguished. The spermatocyte columns lengthen, and later in the season spermatids with nuclei 1.4 μ across occur at the inner tips of the columns. Secondary spermatocytes are probably not seen because they divide rapidly to form

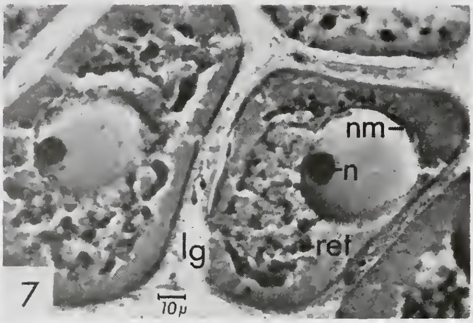

sz
sgn
5μ
lg
2
ctn
ps

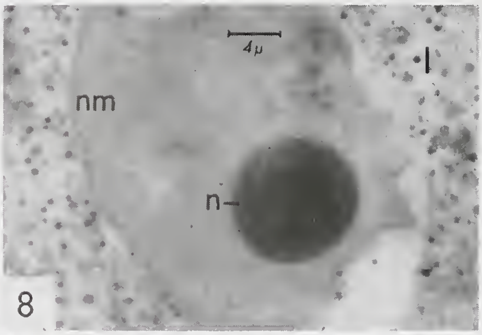

ygc
stn
2μ
3
sz

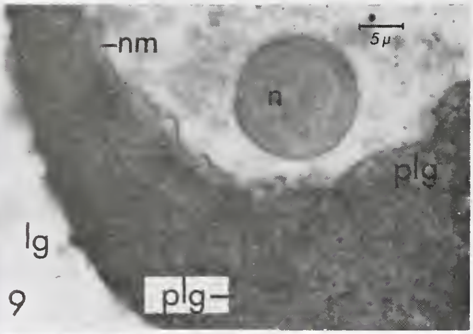

stn
20μ
p
lg
t
scn
4


fn
lg
ocn
10μ
5


15μ
ocn
cg
lg
6
ct


nm
n
lg
ref
7
10μ




nm
5μ
n
plg
lg
9
plg

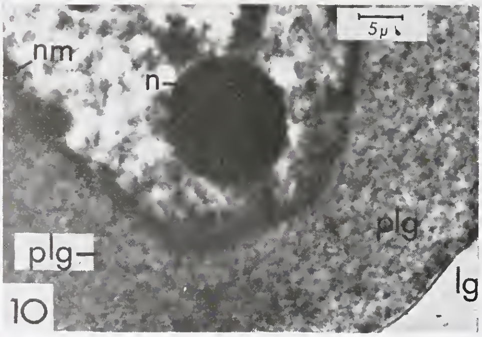

5μ
nm
n
plg
plg
lg
10

FIGURE 2. Section from inner wall of ripe testis of *Pisaster ochraceus*. stained with Prenant's triple stain showing spermatogonia. Symbols used are: ctn, connective tissue cell nucleus;

the spermatids. Spermatids metamorphose into sperm which dissociate from the columns and become evenly distributed throughout the lumen. Spermatogenesis in *Patiria* is essentially similar to the process in *Pisaster*.

The relative abundance of several types of germinal cells during the annual cycle is shown in Figure 1. In both species studied, developing columns of germinal cells without sperm in the lumen were seldom seen, suggesting that the span of time between the formation of primary spermatocytes and the maturation of sperm in any individual animal is relatively short. In Figure 1 the principal spawning period for both species corresponds to the period when the number of oocytes greater than 100 μ in diameter is decreasing.

Many of the histochemical results (Table I) were the same for the testes of both species and they will be described together here. Glycogen or a glycogen-like carbohydrate was present in the cytoplasm of the spermatocytes and to a lesser extent in the spermatids. There is no evidence for glycogen-like carbohydrate in the sperm; small amounts may be present in the spermatogonia. The peritoneal cells also contain granules of glycogen-like carbohydrate early in gametogenesis. In later stages the peritoneal cells are much flattened through stretching as the testis fills with sperm, and the presence of carbohydrate cannot be detected.

Most testicular tissue is consistently negative for all tests for neutral lipid. Irregular yellow granules seen in the peritoneal cells and yellow granules present in the "yellow granule- or globule-containing cells" in the lumen of the testis stain with Sudan black B in frozen sections and in Elftman's technique for phospholipid (though not with hematoxylin at pH 3) suggesting a bound lipid component.

---

ps, perihaemal sinus (genital coelomic sinus); lg, lumen of gonad; sgn, nucleus of spermatogonium; sz, spermatozoa.

FIGURE 3. Edge of lumen of ripe testis of *Patiria,* stained with Harris's hematoxylin, showing the yellow granule- or globule-containing cells. Symbols used are: ygc, yellow granule-containing cells; stn, nuclei of spermatids; sz, spermatozoa.

FIGURE 4. Section from one lobe of testis of *Pisaster,* stained with Prenant's triple stain, showing the spermatogenic columns extending into the lumen. Symbols used are: lg, lumen of gonad; p, peritoneum; scn, nuclei of spermatocytes; stn, nuclei of spermatids; t, tear in tissue, not a sinus.

FIGURE 5. Section from wall region of one lobule of ovary of *Pisaster,* stained with Prenant's triple, showing the small oocytes which remain after spawning and the surrounding follicle cells. Symbols used are: fn, nuclei of follicle cells; lg, lumen of gonad; ocn, nucleus of oocyte.

FIGURE 6. Section from one lobe of ovary of *Pisaster,* sulfated and stained with methylene blue at pH 2.4, showing 0.5 to 1.5 μ granules of carbohydrate which accumulate during growth of the oocyte. Symbols used are: c g, carbohydrate granules, not removed by diastase; ct, connective tissue of ovary wall; lg, lumen of gonad; ocn, nucleus of oocyte.

FIGURE 7. Section of ripe oocytes of *Pisaster,* stained with Prenant's triple stain, showing coarse basophilic reticulum which has receded from the surface of the oocyte. Symbols used are: lg, lumen of gonad; n, nucleolus; nm, nuclear membrane; ret, coarse basophilic reticulum.

FIGURE 8. Section of oocyte of *Patiria,* nearly maximum size, stained with Harris's hematoxylin and oil red 0, showing accumulation of lipid globules in the cytoplasm. Symbols used are: n, nucleolus; nm, nuclear membrane; l, lipid globule.

FIGURE 9. Section of ripe oocyte of *Patiria,* stained with Sudan black B in Elftman's technique for phospholipid, showing phospholipid granules in the cytoplasm. Symbols used are: lg, lumen of gonad; n, nucleolus; nm, nuclear membrane; plg, phospholipid granules.

FIGURE 10. Section of ripe oocyte of *Patiria,* stained with hematoxylin at pH 3 in Elftman's technique for phospholipid. Symbols used are: n, nucleolus; nm, nuclear membrane; plg, phospholipid granules.

Table I

Histochemical results.

	Tests with positive staining
Gonad wall (both sexes)	
Peritoneal cell cytoplasm 0.2 μ granules or diffuse	Periodic acid-Schiff, glycogen sulfation, lithium silver (all three removed by diastase); occasionally standard sulfation
Connective tissue layers	Fast green, standard sulfation, alcian blue; periodic acid-Schiff, glycogen sulfation (both diastase resistant)
Muscle fibers Granules or diffuse	Periodic acid-Schiff (glycogen sulfation *Patiria* only), lithium silver (all three removed by diastase)
Perihaemal sinus coagulum	Periodic acid-Schiff (diastase resistant); gray-brown coloration with lithium silver; pale staining with Sudan III and IV and Sudan black B
Ovary	
Germinal epithelium and follicle cell cytoplasm 0.2 μ granules or diffuse	Periodic acid-Schiff, glycogen sulfation, lithium silver (all three removed by diastase)
Oocytes, 6–10 μ Fine basophilic granules	Harris' hematoxylin, azure A (blue), neutral red; turquoise with glycogen sulfation
Oocytes, 10–100 μ Basophilic reticulum, fine or coarse; basophilic islands or patches	Harris' hematoxylin, azure A (blue), neutral red; turquoise with glycogen sulfation
Granules (0.5–1.5 μ) uniformly scattered in pockets of fine reticulum, later confined to outer edge of cytoplasm or pockets in coarse reticulum	Periodic acid-Schiff, glycogen sulfation, lithium silver (all diastase resistant); standard sulfation
Granules (0.2 μ) mostly in pockets of the reticulum	Periodic acid-Schiff (removed by diastase)
Fine globules	Oil red 0, Sudan III and IV, Sudan black B
Granules (0.3 μ)	Elftman's hematoxylin and Sudan black B
Oocytes, 100–120 μ	
Coarse reticulum or patches	As under 10–100 μ oocytes
Four types of inclusions	As under 10–100 μ oocytes
Jelly layer	Standard sulfation, azure A B-metachromasia, alcian blue
Testis	
Spermatogonia cytoplasm	Pale periodic acid-Schiff and glycogen sulfation (both removed by diastase)
Spermatocytes and spermatids cytoplasm	Periodic acid-Schiff, glycogen sulfation, lithium silver, (all removed by diastase); standard sulfation occasionally; Elftman's hematoxylin and Sudan black B
Spermatozoa	Elftman's hematoxylin and Sudan black B
Cells with yellow granules or globules Yellow granules	Sudan black B (carbowax and paraffin sections)

Phospholipid is present in the developing gametes, but it is difficult to determine whether the positive staining with both parts of Elftman's technique is due to phospholipid in the membranes of cell organelles or to phospholipids stored in the cells.

A *Pisaster* ovary seen in late August (Fig. 5) is filled with growing oocytes, the largest of which are about 25 μ in diameter. The smallest recognizable germinal cells, probably oogonia, are approximately 5 μ across. Flattened follicle cells (Hamann, 1885; Buslé and Delavault, 1968) surround small oocytes in some sections. The smallest recognizable oocytes, approximately 10 μ in diameter, have already accumulated some basophilic cytoplasmic granules, and these granules uniformly fill the cytoplasm of oocytes 25 μ across.

As they further enlarge the oocytes become somewhat tear-shaped because of their close association with the basement membrane. When the swollen end of the oocyte reaches a diameter of about 35 μ the basophilic material forms a fine reticulum in the meshes of which may be found 0.2 μ granules of a carbohydrate possibly removed by diastase (Table I). By the time the oocytes have reached a diameter of approximately 50 μ, yolk granules, each 0.5 to 1.5 μ in diameter, have begun to accumulate in the mesh (Fig. 6). A conspicuous component of these granules is PAS-positive carbohydrate not removed by diastase.

As the oocytes approach a diameter of 100 μ, the fine basophilic reticulum begins to recede towards the interior of the oocyte and becomes a coarse reticulum which then breaks up into isolated patches of darkly staining material (Fig. 7). The outer region of the cytoplasm is filled with the 0.5 to 1.5 μ carbohydrate yolk granules, and a few of these granules also occur in the pockets of the coarse basophilic mesh or between the patches of basophilic material.

In the pockets between strands of the coarse mesh there are also numerous smaller granules, 0.2 μ in diameter, which contain a carbohydrate which is perhaps removed by diastase and therefore glycogen; the judgment is a difficult one because of the number of intensely-staining carbohydrate yolk granules also present. The ripe oocyte is about 120 μ in diameter, contains a nucleus approximately 40 μ in diameter, and a nucleolus 12 to 14 μ in diameter.

Even in the ripe ovary small oocytes 10 μ in diameter are present along the wall, and as Mauzey (1966) also suggests, these oocytes will probably be left after spawning to mature in the succeeding breeding cycle. Once the ripe oocytes have been spawned, one occasionally sees in the lumen cells with yellow granules or globules similar to those in the testes. Irregular yellow granules similar to those seen in the peritoneum of the testes are also seen in the peritoneal cells of the ovary.

The development of the oocyte in *Patiria* is essentially as described in *Pisaster*.

In addition to the two types of carbohydrate granules mentioned above, the cytoplasm of the oocytes of *Patiria* and *Pisaster* also contains neutral lipid droplets (Fig. 8) and phospholipid granules (Figs. 9 and 10); there also seem to be granules unusually rich in protein, though the heavy background staining makes this conclusion only tentative. Whether the protein, carbohydrate and phospholipid occur in different granules or are combined or layered in one or two types of granules or platelets cannot be determined from available materials.

Generally in March or April (though sometimes as early as January) a clear extracellular layer appears around the fully grown oocytes of *Patiria* and *Pisaster*.

The staining affinities of this layer, which probably corresponds to the jelly layer of embryologists, are those of an acid mucopolysaccharide, possibly weakly sulfated.

Early in the breeding cycle the perihaemal sinus of the ovary and testis in both *Pisaster* and *Patiria* often contains carbohydrate substance not removed by diastase. In addition the material in the sinus occasionally stains diffusely with neutral lipid dyes.

Gonad index and histochemistry of gonads in starved animals

Starvation of specimens of *Pisaster* and *Patiria* is unquestionably associated with a reduction in the number of gametes produced, as reflected in the gonad index determinations (Nimitz, 1971, Table III). Specimens of *Pisaster* collected on December 15, 1964, and starved for fourteen months until February 7, 1966, had an average gonad index of 0.33 ± 0.25 (standard deviation) in contrast to field animals collected in February which had an average gonad index of 5.4 ± 3.3. Some of the starving animals had spawned fairly copiously in the tanks at the Hopkins Marine Station on August 15, 1965 (Giese, personal communication). The specimens of *Pisaster* starved for twenty months until July 27, 1966 had an average gonad index of 0.15 ± 0.02 as opposed to an index of 3.2 ± 3.02 for animals freshly collected from the field. In one sense, using July field animals which had spawned as controls, does not show the extent of the impact of twenty months of starvation as well as it might. The starving animals had presumably not spawned in 1966 and thus would be more appropriately compared to the March field animals which had not spawned and which had an average index of 15.1 ± 4.5. Unfortunately the "field animals" in the above study could not be taken from the same population from which the starving animals had come, which may cast some doubt on the validity of using them as controls (Nimitz, 1971).

In *Patiria* the starving animals presumably had an initial average gonad index of 2.8 ± 3.1 as determined from the gonad index determination of a sample taken from the field at the same time as the animals placed under starvation. The gonad index of the starving animals shrank to an average of 1.8 ± 1.3 in May after eight months of starvation, as compared to May field animals with an average index of 4.0 ± 1.7.

Only two testes of starved *Pisaster* were successfully processed. The testis of an animal starved fourteen months contained recognizable spermatogonia with a few granules of glycogen-like carbohydrate. The germinal epithelial cells and peritoneal cells of the specimen starved fourteen months and of the one starved twenty months contained moderate numbers of carbohydrate yolk granules. The specimen starved fourteen months showed neutral lipid globules in the germinal epithelial cells, and similar globules appeared in the peritoneal cells of the one specimen starved twenty months. Normal testes do not show neutral lipid droplets in either site. Numerous cells containing the yellow granules or globules were present in the lumen. These are possibly engaged in breaking down the germinal cells and/or gathering up the products of breakdown for transport and use elsewhere.

In the single ovary processed for carbohydrates from specimens starved for fourteen months, the largest oocytes were 24 μ in diameter and lacked the carbohydrate yolk granules. The peritoneal cells, germinal epithelium, and follicle cells were moderately rich in 0.2 μ carbohydrate granules. Tissues from two specimens were processed successfully through lipid techniques, and some neutral lipid was present in the germinal epithelium and follicle cells.

Two ovaries from animals starved twenty months showed 0.2 μ granules of carbohydrate present in the germinal epithelial and peritoneal cells. In tissue processed for lipids there were found some oocytes 7 μ in diameter and 18 μ long with tiny lipid globules stainable with Sudan black B. Among the animals which had spawned after eight months of starvation and then starved an additional twelve months, no new cycle of gonadal growth had been initiated by the time of sacrifice (or if new germinal cells had been produced during the last year of starvation they had subsequently been resorbed).

The ovaries of *Patiria,* starved from late August to May, all showed a reduction in the number of large oocytes, and in three specimens, it appeared that the oocytes were being broken down. The large oocytes that remained contained fine droplets of neutral lipid, and in the areas of the ovary where resorption was occurring there were larger droplets of lipid in masses of debris near the ovary walls. Among the debris there appeared to be numerous isolated nucleoli, some still basophilic, others colorless, of various sizes. The oocytes stained strongly with carbohydrate techniques, but the staining appeared diffuse, not associated with discrete granules characteristic of normal oocytes. The other germinal epithelial cells (*i.e.*, would-be follicle cells and oogonia) and peritoneal cells stained strongly for carbohydrate, and the germinal epithelial cells may contain some neutral lipid. The positive staining for nutrient materials in the ovary is not surprising if the oocytes are being broken down. It is reasonable to expect that nutrients released during the breakdown would be taken up into the adjacent germinal epithelial cells.

The testes of starved *Patiria* contained fewer sperm than usual, but the normal spermatogenic cycle had apparently been carried to completion, since the lumen of each testis contained only ripe sperm; no columns of developing cells were seen. Spermatogonia were seen in moderate number in the germinal epithelium. The cells containing yellow granules or globules were unusually abundant in the lumen. There was no evidence of neutral lipid present in slides stained with oil red 0, but in Sudan black B-stained slides, the sperm appeared to contain tiny droplets of lipid in the thin layer of cytoplasm of the head region. This is possibly phospholipid, given the negative reaction with oil red 0. It is puzzling that lipid should show up as droplets in sperm of starved animals and not in normal ones, but this could have resulted from improved conditions for studying the sperm when they were not so crowded as usual. In three out of four specimens there was a diffuse staining in the testes for carbohydrates in both the germinal epithelial and peritoneal cells. All the carbohydrate staining, including that of connective tissue, was removed by pancreatin, but through human error no slides of starved *Patiria* gonad were run with amylase or diastase incubation.

DISCUSSION

Farmanfarmaian *et al.* (1958) earlier reported spawning of *Pisaster ochraceus* in April 1954 and 1955 and March 1956, and Greenfield (1959) in June 1958. Farmanfarmaian *et al.* (1958) reported spawning of *Patiria* in June and July 1955 and Lawrence (1965) in June 1963 and January 1964.

Giese's (1966b) biochemical data indicate that the gonads in both sexes of specimens of *Pisaster* of low gonad index contain lipid amounting to about 5% of the dry weight. In the male there is an increase to 8% lipid (dry weight) in animals of intermediate gonad index, while animals with high index are poor in lipid (3.5 ± 5.2%). If one considers that about 5% of the lipid in tissue is structural lipid (Giese, 1966a), significant neutral lipid stores would not be expected in the testis. Allen (1964) indicates that 70% of testis lipid in *Pisaster ochraceus* is polar lipid, chiefly phospholipid. The histochemical findings of this present study support strongly both the scarcity of neutral lipid and the abundance of phospholipid.

The testes of *Patiria* contain roughly 18% lipid (Giese, 1966b). This lipid is not demonstrable as neutral lipid by the histochemical procedures used in the present study and is presumably phospholipid. The higher lipid content in the testes of *Patiria* than in testes of *Pisaster* may relate to the greater abundance in this species of the cells containing the yellow granules (globules?) which stain intensely with Sudan black B.

The ovary of *Pisaster* increases in per cent of dry weight of lipid from approximately 5 to 35% (Giese, 1966b) at the same time it is increasing in mass preparatory to breeding, and Allen (1964) has found that polar lipid, largely phospholipid, accounted for 70% of the lipid in immature gonads of *Pisaster* and for 55% of the lipid in ripe ovaries. The biochemically detected increase of lipid in the ovary of *Pisaster* correlates with the histochemical observations of neutral lipid droplets and phospholipid granules accumulating in great abundance in the oocytes.

The *Patiria* ovary averages about 20% lipid (Giese, 1966b). Work with other sea stars indicates total lipid varying with the reproductive cycle but always with high levels of polar lipid in the ovaries and low total lipid with high proportion of polar lipid in the testes (Pearse and Giese, 1966; Akino, Shimojo, and Sasaki, 1970; Lawrence, 1973).

Allen and Giese (1966) have found that the rate at which labeled precursors are incorporated into lipid in the gonads of *Pisaster ochraceus* increases from November to February. The increase in lipogenic rate in the gonads occurs about the time the hepatic caeca begin to shrink and probably at their expense.

A glycogen-like material accounts for 0.43% of the dry weight of the testis in *Pisaster* of low gonad index and this decreased to less than 0.1% in individuals of high gonad index. *Patiria* testis averages about 2% dry weight of glycogen-like material (Giese, 1966b). The carbohydrate is histochemically detectable in the cytoplasm of the spermatogonia and spermatocytes, though not in the sperm, in both *Pisaster* and *Patiria*.

The ovaries of *Pisaster* of low gonad index show a small amount of glycogen-like material in the germinal epithelial cells, peritoneum, and possibly also in the small oocytes, according to histochemical results. Such individuals were found by Giese (1966b) to have a glycogen-like carbohydrate content of 0.4%. During oogenesis the biochemically detectable glycogen-like carbohydrate is reduced to 0.25%. The *Patiria* ovary contains about 0.6% glycogen-like carbohydrate. The occurrence of histochemically detectable glycogen-like material in the larger oocytes is uncertain, because the existence of contrast between diastase or amylase-treated slides and the controls is difficult to judge. An abundance of carbohydrate resistant to diastase accumulates as 0.5–1.5 μ yolk granules in the oocytes during growth.

Mauzey (1966) has also found this carbohydrate refractory to amylase in his studies of *Pisaster,* and Chia (1968) found a similar refractory carbohydrate associated with protein and lipid in the yolk platelets of the oocytes of *Leptasterias hexactis.* The failure of the amylase or diastase to digest this carbohydrate does not rule out the possibility that it is glycogen, since glycogen complexed with protein might be protected from digestion.

J. M. Lawrence (unpublished data cited in Giese, 1966b) has found 15 to 20 mg of lipid per 100 g of body fluid in *Pisaster ochraceus,* 6.5 to 7.3 mg in *Patiria.* Glucose in the body fluid (A. L. Lawrence, unpublished data cited in Giese, 1966b) amounts to roughly 0.25 to 0.29 mg per 100 g in *Pisaster,* and 0.20 mg in *Patiria,* yet the total carbohydrate of the body fluid varies from 0.70 mg in *Pisaster* of low gonad index to 1.84 mg in specimens of of high gonad index. In *Patiria* the total carbohydrate ranged from 0.78 to 0.87 mg per 100 g of body fluid. Since glucose accounts for only a fraction of the total carbohydrate, other sugars and/or sugar polymers are probably present in the sea stars body fluids. The histochemical staining for carbohydrate in the perihaemal sinus of *Pisaster,* observed also by Mauzey (1966), probably represents carbohydrate polymers since simple sugars are assumed to wash out of tissue sections during histochemical processing.

The development of large amounts of vesicular tissue after spawning, as described for *Asterina gibbosa* by Bruslé, Tereygeol, and Delavault (1970), has no parallel in *Pisaster* and *Patiria.* This may correlate with the presence of ample storage space in the pyloric caeca, which do contain significant stores of lipid and amylase-refractory carbohydrate and which do, a least in *Pisaster,* show cyclic changes in size correlating with the reproductive cycle (Nimitz, 1971, Figs. 7 and 8). It seems probable that in *Pisaster* and *Patiria* nutrients used in gametogenesis are derived from reserves in the pyloric caeca.

Parallel to findings in this present study, Crump (1971) has shown a striking impact of food availability on reproductive potential in *Patiriella regularis,* since the average gonad index of animals fed crabs to satiety was 26.13 ± 10.69 as compared with the average gonad index of 1.36 ± 1.45 in animals starved for 44 weeks. (Crump defined GI as the ratio of the volume of the gonad to the body weight after removal of gonads and caeca, × 100.) The gonads of *Patiriella* starved for 44 weeks likewise contained only immature gametes at a time when control animals showed gonads ready for spawning.

The author wishes to acknowledge gratefully the assistance of Elizabeth Saghy, Penny Livermore, Charlotte Elmore, Robert Spies, Margaret Probert, Charlotte Whittaker, Sister M. Pamela, O. P., Sister Diane Smith, O. P., Terry Kremesec, and Gladys Charshaf in collecting and processing the sea stars and preparing the photograph prints and the manuscript. She also wishes to thank Sister M. Frieda Ward, O. P., and Dr. Nicholas Holland for their critical reading of the manuscript.

SUMMARY

1. *Pisaster ochraceus* has an annual reproductive cycle in which the gonads increase in size rapidly from October or November and reach a maximum in March to May, after which spawning occurs. In *Patiria miniata* the gonadal cycle is less

pronounced and less regular. In the course of this study, animals spawned in July 1965, May 1966, and May 1967.

2. Histochemical techniques indicate that in both species glycogen or a glycogen-like carbohydrate occurs in the germinal epithelium and the follicle cells of the female, and in the spermatogonia and primary spermatocytes. A storage carbohydrate which is not removed by diastase or amylase is abundant in oocytes in the form of yolk granules 0.5 to 1.5 μ in diameter.

3. Neutral lipid droplets and phospholipid granules are abundant in all oocytes but the smallest. In the testes, lipid droplets are seen only after prolonged starvation.

4. In both species prolonged starvation results in failure of the gonads to achieve their normal size increase. Such gametes as are seen in starving specimens appear histochemically normal in some instances; in other cases they seem to be undergoing breakdown.

5. The histochemical results concerning nutrient reserves of the gonads are generally in agreement with the biochemical findings of earlier workers.

LITERATURE CITED

Akino, T., T. Shimojo and T. Sasaki, 1970. Studies on visceral lipids of starfish. I. Lipid components of various organs. *Sapporo Med. J.*, **37**: 182–192.

Allen, W. V., 1964. Lipogenesis in the sea star, *Pisaster ochraceus. Ph.D. dissertation, Stanford University*, 173 pp. (*Diss. Abstr.*, **26**: 454; order number 65–6266.)

Allen, W. V., and A. C. Giese, 1966. An *in vitro* study of lipogenesis in the sea star *Pisaster ochraceus. Comp. Biochem. Physiol.*, **17**: 23–38.

Bruslé, J. and R. Delavault, 1968. Recherches sur la cytodifférenciation des gamètes chez un hermaphrodite fonctionnel: *Asterina gibbosa.* Ultrastructure des ovogonies et des ovocytes en préméiose, *C. R. Hebd. Seanc. Acad. Sci. Paris,* **266**: 21–23.

Bruslé, J., G. Téreygeol and R. Delavault, 1970. Le tissu vésiculeux dans les gonades d'*Asterina gibbosa.* Données histochimiques et ultrastructurales. *Boll. Zool.*, **37**: 37–49.

Chia, F. 1968. Some observations on the development and cyclic changes of the oocytes in a brooding starfish, *Leptasterias hexactis. J. Zool. London,* **154**: 453–461.

Crump, R. G., 1971. Annual reproductive cycles in three geographically separated populations of *Patiriella regularis* (Verrill), a common New Zealand asteroid. *J. Exp. Mar. Biol. Ecol.*, **7**: 137–162.

Davis, H., 1971. The gonad walls of Echinodermata: a comparative study based on electron microscopy. *Master's thesis, University of California, San Diego,* San Diego, California, 90 pp.

Delavault, R. and J. Bruslé, 1968. Recherches sur la cytodifférenciation des gamètes chez un hermaphrodite fonctionnel: *Asterina gibbosa.* Ultrastructure des cellules de la lignée spermatogénétique et comparison spermatogonies-ovogonies. *C. R. Hebd. Seanc. Acad. Sci. Paris,* **266**: 710–712.

Farmanfarmaian, A.. A. C. Giese, R. A. Boolootian and J. Bennett, 1958. Annual reproductive cycles in four species of west coast starfishes. *J. Exp. Zool.*, **138**: 355–367.

Feder, H., 1956. Natural history studies on the starfish, *Pisaster ochraceus* (Brandt, 1835) in the Monterey Bay area. *Ph.D. dissertation, Stanford University,* 294 pp. (*Diss. Abstr.*, **17**: 726; order number 20,446).

Giese, A. C., 1966a. Lipids in the economy of marine invertebrates. *Physiol. Rev.*, **46**: 244–298.

Giese, A. C., 1966b. On the biochemical constitution of some echinoderms. Pages 757–796 in R. A. Boolootian, Ed., *Physiology of Echinodermata.* Interscience, New York.

Greenfield, L. J., 1959. Biochemical and environmental factors involved in the reproductive cycle of the sea star *Pisaster ochraceus* (Brandt). *Ph.D. dissertation, Stanford University,* 143 pp. (*Diss. Abstr.*, **20**: 3049; L. C. Card. No. Mic. 59–6890).

Greenfield, L., A. C. Giese, A. Farmanfarmaian and R. A. Boolootian, 1958. Cyclic biochemical changes in several echinoderms. *J. Exp. Zool.,* **139**: 507–524.

Hamann, O., 1885. *Beiträge zur Histologie der Echinodermen.,* Heft 2 - Die Asteriden anatomisch und histologisch untersucht. Jena, Fisher.

Holland, N. D. and A. C. Giese, 1965. An autoradiographic investigation of the gonads of the purple sea urchin (*Strongylocentrotus purpuratus*). *Biol. Bull.,* **128**: 241–258.

Lawrence, J. M., 1965. Lipid levels in the body fluid, blood, and tissues of some marine molluscs and echinoderms in relation to nutritional and reproductive state. *Ph. D. dissertation, Stanford University,* 105 pp. (*Diss. Abstr.,* **27**: 331B; order number 66-6362).

Lawrence, J. M., 1973. Level, content, and caloric equivalents of the lipid, carbohydrate, and protein in the body components of *Luidia clathrata* (Echinodermata: Asteroidea: Platyasterida) in Tampa Bay. *J. Exp. Mar. Biol. Ecol.,* **11**: 263–274.

Mauzey, K. P., 1966. Feeding behavior and reproductive cycles in *Pisaster ochraceus. Biol. Bull.,* **131**: 127–144.

Nimitz, Sr. M. Aquinas, 1971. Histochemical study of gut nutrient reserves in relation to reproduction and nutrition in the sea stars, *Pisaster ochraceus* and *Patiria miniata. Biol. Bull.,* **140**: 461–481.

Pearse, J. S., and A. C. Giese, 1966. The organic constitution of several benthonic invertebrates from McMurdo Sound, Antarctica. *Comp. Biochem. Physiol.,* **18**: 47–57.

Walker, C. W., 1974. Studies on the reproductive systems of sea stars. I. The morphology and histology of the gonad of *Asterias vulgaris. Biol. Bull.,* **147**: 661–677.

Reference *Biol. Bull.*, **151**: 370-385. (October, 1976)

CYTOTOXIC EFFECTS OF THE HERBICIDE 3-AMINO-1,2,4-TRIAZOLE ON *DAPHNIA PULEX* (CRUSTACEA: CLADOCERA)

T. WAYNE SCHULTZ[1] AND JOHN R. KENNEDY

Department of Zoology, University of Tennessee, Knoxville, Tennessee 37916

Chemical control of nuisance plants is usually the most economical method available (Meyer, 1966). This has made the use of herbicides a popular technique for control of plants in aquatic environments. However, the introduction of herbicides into aquatic systems has been shown to have an adverse effect on non-target organisms such as aquatic invertebrates. Thus Walker (1964, 1965) and Cowell (1965) have reported drastic reductions in pond invertebrate population levels following application of herbicides.

One of the more commonly used herbicides is Amitrole (3-amino-1,2,4-triazole). It was first introduced as a herbicide in 1954 under the commercial name of Weedazole by Amchem Products, Incorporated. Commonly referred to as aminotriazole, the compound is a white crystalloid of 84.1 molecular weight and 150–153° C melting point. This heterocyclic nitrogen compound with its highly stable S-triazole nucleus is quite soluble in water (Mason, 1969).

Several authors have undertaken herbicide bioassay research on aquatic invertebrates (for review see Bunting and Robertson, 1975). In the past these papers have dealt primarily with the acute effects which these chemicals have on selected species of the cladoceran *Daphnia*. Crosby and Tucker (1966), using first instar organisms, investigated the toxicity of 16 herbicides on *Daphnia magna* Straus. They found in 26-hour acute static tests at 21° C the median immobilization concentration (IC50) of amitrole to be 23 ppm. This compares favorably with the work of Findley (1969), who in using aminotriazole, an amitrole formulation with 50% active ingredient, found that 0-24 hr old *D. magna* had an IC50 of 53.5 ppm in 24 hour acute static tests at 20° C. She also found a drastic reduction in IC50 value, to 3.55 ppm, when the test period was extended to 48 hr (Findley, 1969). Chronic bioassay studies have shown that aminotriazole in concentrations as low as 3.5 ppm retarded growth and decreased fecundity. Findley (1969) found that over a ten day period the carapace length was significantly shorter in animals reared in 3.5 ppm aminotriazole. She further noted that 80% of the adults reared in 3.5 ppm aminotriazole solution failed to produce eggs (Findley, 1969). Bunting and Robertson (1975), extending the work of Findley, found *Daphnia pulex* Leydig to be more sensitive to aminotriazole than *D. magna*. They determined that 0–12 hr old *D. pulex* exposed under static conditions for 48 hr at 20° C reached median survival concentration at 15.5 ppm. They further note that *D. pulex* is more sensitive to reproductive damage than is *D. magna*. Temperature affects the rate at which the herbicide acts at 15 and 20° C. Thus, to obtain similar rates of survival it was necessary to double the aminotriazole concentration at 15° C (Bunting and Robertson, 1975).

---

[1] Present address: Biology Division, Oak Ridge National Laboratories, Oak Ridge, Tennessee 37830

This study was undertaken to examine the cytotoxic effects of the herbicide 3-amino-1,2,4-triazole in concentrations of 10 ppm (0.1 mg/ml) on the common water-flea *Daphnia pulex,* and to examine possible modes of entry into the animal.

MATERIALS AND METHODS

The original stock culture of *Daphnia pulex* Leydig, secured from Turtox National Biological Supply, was identified using the key and description of Brooks (1957). A single adult from this population was randomly selected and cloned to provide all the animals (parthenogenetic females) used throughout the course of these studies.

The *Daphnia* was fed following the modified procedures of Dewey and Parker (1964) and Findley (1969) using a suspension of *Scenedesmus obliquus* (Indiana University Culture Collection, Starr, 1964), along with Fleishmann's dry yeast. The water was filtered once through No. 1 Whatman filter paper and stored to be used as needed. Chemical analyses were performed each time the water was filtered using a Hach DR-EL (Hach Chemical Co., Ames, Iowa) direct-reading portable kit (Table I). The pH was measured using a Corning Model 7 meter and was found to be between 7.0–8.2, well within the normal pH range of 5.8 and 9.2 for *Daphnia* (Lowndes, 1952).

The specimens of *S. obliquus* were grown in 1.0 liter Erlenmeyer flasks containing 500 ml of sterile filtered pond water to which had been added 10 ml of Alga-Gro concentrate (Carolina Biological Supply Co). The algal cultures were maintained at 24 ± 1° C in a 16 hr photoperiod of Grow-lux (Fisher Scientific, Atlantic, Ga.) fluorescent lighting. The algae were allowed to grow until the cultures were a dark green in color giving a Spectronic 20 reading of greater than 0.60 absorbance at 680 mμ prior to use.

Young daphnids were reared and collected by placing 5–8 adult *Daphnia* in each of several 100 ml beakers containing 75 ml of filtered pond water with 5 ml of suspended *Scenedesmus* culture added as food. The beakers were placed in an environmental chamber at 24 ± 1° C with a 16 hr Grow-lux flourescent lighting photoperiod imposed. At given time intervals, adults were removed by pipette and placed in freshly prepared beakers. All glassware used was chemically cleaned (Findley, 1969).

All the tests conducted were static in design. A stock solution of herbicide was prepared by dissolving 0.5 gram technical grade (100% active ingredient) 3-amino-S-triazole (Amchem Products, Inc., Ambler, Pennsylvania) in 500 ml of the filtered pond water. From the stock solution a lethal concentration of 10

TABLE I

Chemical analyses of pond water.

Analysis for	Mean (ppm)	Range (ppm)
Alkalinity (total)	84.2	70–100
Calcium hardness	70.0	50–80
Magnesium hardness	22.8	10–40
Total hardness	90.7	85–100
Iron (total)	0.08	0.06–0.10

ppm (0.1 mg/ml) was prepared with filtered pond water. The above concentration was selected based on the field use of amitrole as noted by Crosby and Tucker (1966). The final concentration solution was prepared fresh daily. The stock solution was discarded and fresh solution prepared every five days.

Individuals in the age range of 0–24 hr (post brood chamber release) were selected for study because Breukelman (1932) and Sanders and Cope (1966) found young animals to be more susceptible to herbicides than older individuals.

Ultrastructure studies

Acute lethal experiments for examination of cytotoxic effects were conducted in 8 dram vials. Each replication consisted of 24 crustacean (0–24 hr post brood pouch release) placed in individuals vials of 25 ml solution of 3-amino-1,2,4-triazole (10 ppm) to which had been added 1 ml of suspended *Scenedesmus* culture. Each vial was checked hourly after the initial 12 hr period and any immobile individuals removed and fixed for electron microscopy. An animal was considered to be immobile if, upon tactile stimulation with a glass pipette, it did not swim away. Immobilization rather than death of the daphnids was selected because of the difficulty in ascertaining when death occurs.

In addition, time sequence exposure was performed on 0–24 hr old individuals. Approximately 60 specimens of *Daphnia* were placed into 150 ml of a 3-amino-1,2,4-triazole solution (10 ppm) in a wide-mouth 250 ml Erlenmeyer flask to which had been added 10 ml of suspended *Scenedesmus* culture. At 6, 9, 12 and 15 hr after initiation of the experiment approximately ten animals were removed and fixed for examination with the electron microscope.

Specimens of *Daphnia* were fixed for electron microscopy by immersing them in 1% osmium tetroxide in 0.2 M phosphate buffer at pH 7.4. After fixation at room temperature for two hr, the animals were rinsed in phosphate buffer at room temperature for one hr. The daphnids were dehydrated in a cold graded ethanol series followed by several changes of propylene oxide prior to infiltration and flat embedding in Epon 812.

Sections were cut using a diamond knife and a Porter-Blum (MT-1) ultramicrotome and picked up on naked 300-mesh and Formvar coated 150-mesh copper grids. Staining was accomplished with uranyl acetate (Watson, 1958) and lead citrate (Venable and Coggeshall, 1965). Electron photomicrographs were prepared using an RC EMU 3H or Zeiss EM 9S transmission electron microscope.

Acute static bioassay

In an attempt to more critically ascertain the relationship between the timing of herbicide toxicity and the molt cycle, 0–2 hr old daphnids were employed. Three types of experiments were conducted: first, effects of continuous exposure over a 24 hr period on 0–2 hr old daphnids; secondly, effect of shorter term exposure to different age groups during the first 24 hr growth; and thirdly, effects of temperature.

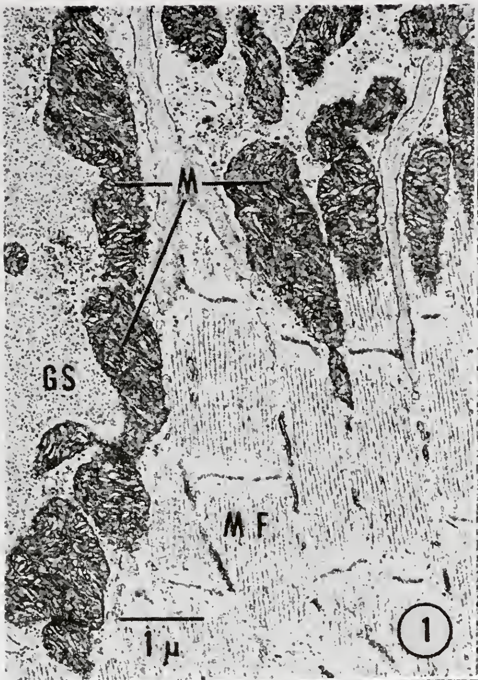
In all studies individuals were placed in 8 dram vials filled with 25 ml of 3 amino-1,2,4-triazole (10 ppm at 24° C) with 1 ml of *S. obliquus* suspension added as a food source. The organisms were examined at half hour intervals to establish the time of onset of immobility. Controls without herbicide were run simultaneously and experimental samples were corrected by subtracting the number of immobilized control animals from the number of immobilized herbicide exposed animals in corresponding samples. The best line for the data was fitted by least squares method of linear regression and the regression coefficient tested by one-way analysis of variance to see if it differed significantly from zero.

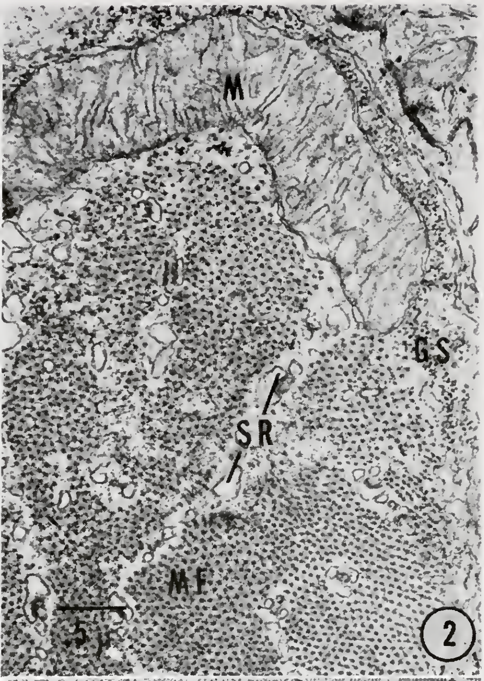
Results

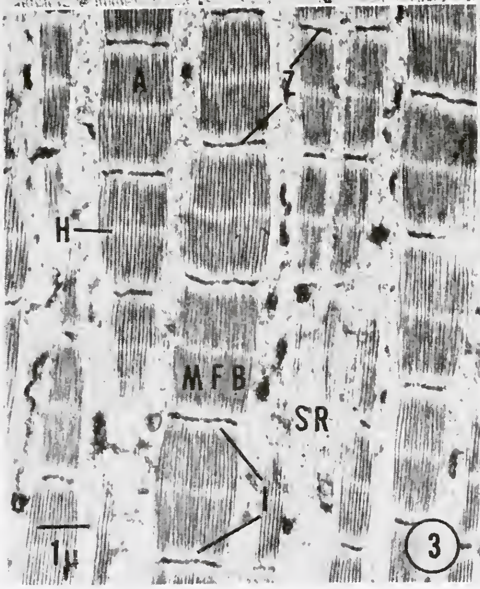
Cytotoxicity experiments

Several deviations from normal fine-structure were evident in daphnids exposed to 3-amino-1,2,4-triazole (10 ppm) until immobilization. The most consistent of these alterations in cell structure was seen in the mitochondria, especially those of the more metabolically active tissue such as the body musculature. The body musculature of *Daphnia* has been extensively studied with the light microscope (Binder, 1931). The striated muscle of *D. pulex* resembles that described in the copepod (Bougligand, 1962). In longitudinal section the normal fine structure shows the typical striations of skeletal muscle, with the "A" and "I" bands clearly distinguishable and with an "H" band and a "Z" line bisecting them, respectively (Figs. 1 and 3). The sarcomeres are short and of uniform length, 3 μm in the relaxed state, with the myofibrils varying in thickness and separated by varying amounts of sarcoplasmic reticulum (Figs. 1–4) and mitochondria. Individual fibers of the body muscularis vary markedly in their fine structure especially in regards to size of fibrils, amount of sarcoplasmic reticulum, number and size of mitochondria, and amount of glycogen granules. Some cells, "Fibrillenstruktur" fibers (Hoyle, 1967), have extensive sarcoplasmic reticulum (SR). The myofilaments (MF) are separated into bundles and the myofibrils (MFB) each completely enclosed by the sarcoplasmic reticulum (Figs. 3 and 4). Other cells are "Felderstrucktur" fibers (Hoyle, 1967) and have little sarcoplasmic reticulum (Figs. 1 and 2). Intermediate forms are also observed in *Daphnia.* Muscle of the appendages (Fig. 3) is characterized by an extensive sarcoplasmic reticulum (SR) to myofibril ratio. The muscle fibers are multinucleate with the nuclei being peripheral in location and circular in section. Also located peripherally are large numbers of mitochondria (M) and interspersed extrafibrillar glycogen stores (Figs. 1–4; GS). These muscle mitochondria are polymorphic in shape (Figs. 1–4). For the most part they are much larger than their counterparts found anywhere else in *Daphnia,* being up to 1.2 μm in length. Each mitochondrion possesses a large number of closely packed inner membranes, or cristae, and a moderately dense ground matrix all enclosed by a continuous other membrane.

Upon immobilization of *Daphnia* the muscle mitochrondria examined expressed various degrees of alteration from normal to lysing (Figs. 5–7). Not all muscle cells within a single organism were affected nor were all mitochondria within a single fiber affected equally (Fig. 7). However, for the most part mitochondria of all cells of a single muscle were altered to some degree structurally. The most common alteration observed was folding (arrow) of the outer mitochondrial







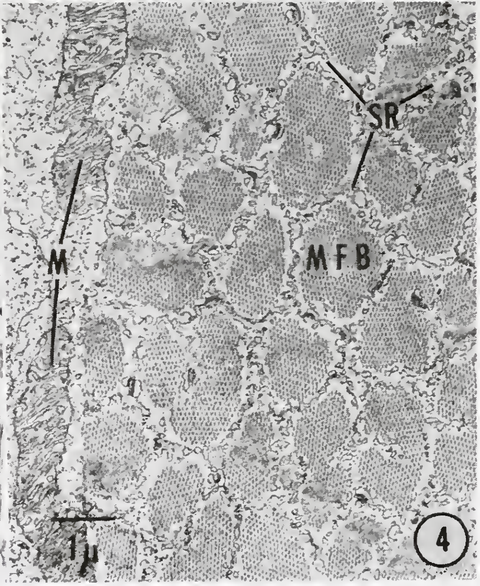


FIGURE 1. An oblique section through the edge of a normal "Felderstrucktur" muscle cell. Numerous large mitochondria (M) are located peripheral to the myofilaments (MF). Interspersed among the mitochondria are extensive glycogen stores (GY).

FIGURE 2. A transverse section through a normal "Felderstrucktur" muscle cell. Note the myofilaments (MF) are not seperated into descrete bundles by the sarcoplasmic reticulum (SR). A peripheral mitochondrion (M) and glycogen stores (GS) can also be observed.

membrane (Figs. 5–7). This was accompanied by organelle swelling and a reduction in electron density of the ground matrix and number of cristae (Fig. 5). Often observed was more extensive mitochondrial degradation characterized by gross shape alterations accompanied by drastic reduction in cristae number and matrix content (Fig. 6). Moreover, a few mitochondria were lysed (Fig. 11, LM). A small percentage of the muscularis mitochondria within a cell otherwise altered by 3-amino-1,2,4-triazole were observed to be only slightly affected, if at all (Fig. 7). Similar mitochondrial damage was observed in other cell types, though much less regularly. This was most often noted in the hypodermis (Fig. 8) and eye (Fig. 9) and was usually characterized by mitochondrial swelling. For comparison, inserts contain normal mitochondria from comparable regions of control animals.

In addition to mitochondrial alteration other cytotoxic effects were observed. These included disarrangement of the fibrils and myofilaments (MF) of the muscle cell, with the sarcoplasmic reticulum (SR) showing a concomitant swelling (Figs. 10, 11). The median compound eye often showed swelling and disassociation of the membranes enclosing the pigment granules (Fig. 9, PG). The least affected organs, at least structurally, were the midgut and ovaries. The latter showed only slight mitochondrial damage, while the former occasionally expressed alteration in cell shape and microvilli.

The cytotoxicity observed in *D. pulex* exposed to 3-amino-1,2,4-triazole (10 ppm) until becoming immobile is the same for 0–24 hr old and adult animals. While there was a wide range of structural alterations expressed by a variety of cell types, a general trend did appear. Mitochondrial damage was by far the most consistent structural change noted in the test animals and was often (50% of the time) the sole alteration observed. At no time during the course of the investigation were additional cytotoxic effects such as myofibril, sarcoplasmic reticulum or microvilli damage noted without accompanying mitochondrial swelling.

Mobile 0–24 hr old daphnids, exposed to the herbicide (10 ppm at 24° C) for up to 15 hr, showed no cell or organelle damage regardless of length of exposure. Reproductive-age specimens of *D. pulex* were also exposed to 3-amino-1,2,4-triazole (10 ppm, 24° C) until immobilization. Upon examination cytotoxic alterations comparable to those in 0–24 hr old animals were observed.

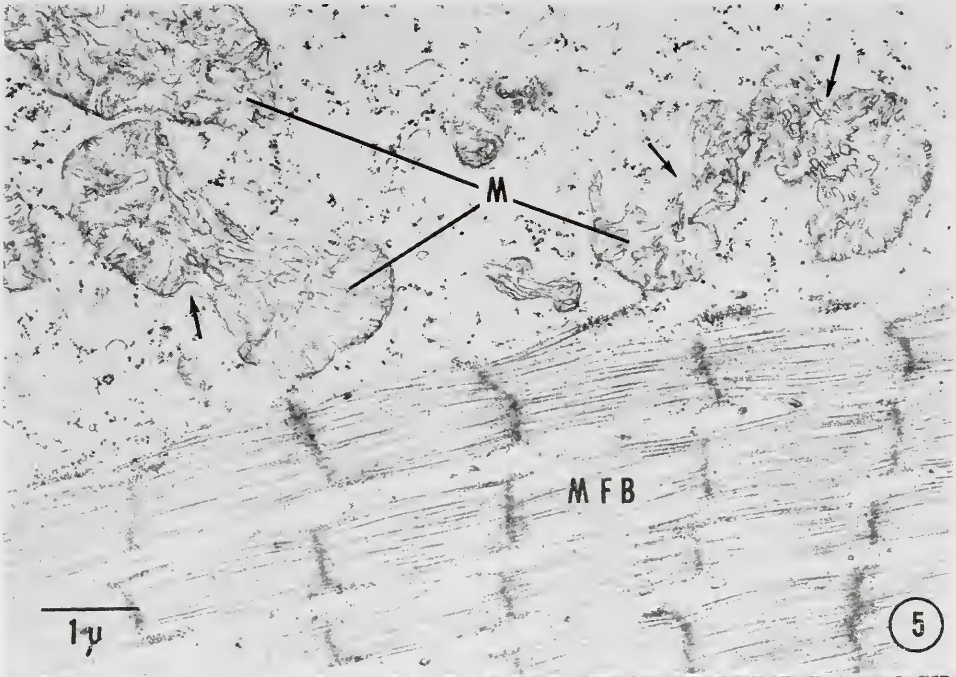
Acute static bioassay

During the course of the acute static tests and exposure for cytotoxicity studies (10 ppm 3-amino-1,2,4-triazole at 24° C) the 0–24 hr old and adult *D. pulex* expressed a change in behavior pattern. Prior to the onset of immobilization a decrease in the rate and efficiency of the antennal stroke or swimming move-

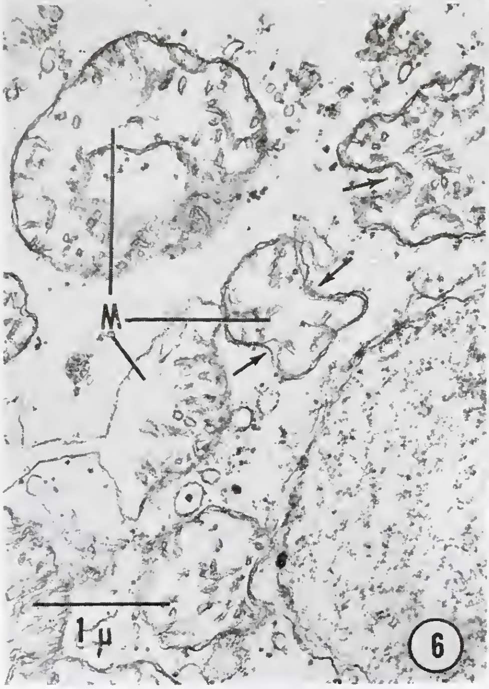
---

FIGURE 3. A longitudinal section through a normal "Fibrillenstrucktur" muscle cell. The myofibrils (MFB) are parallel and generally of equal width. They are separated by extensive sarcoplasmic reticulum (SR). Note the typical banding pattern of an "A" band (A) and "I" band (I) bisected by a "H" band (H) and "Z" line (Z), respectively.

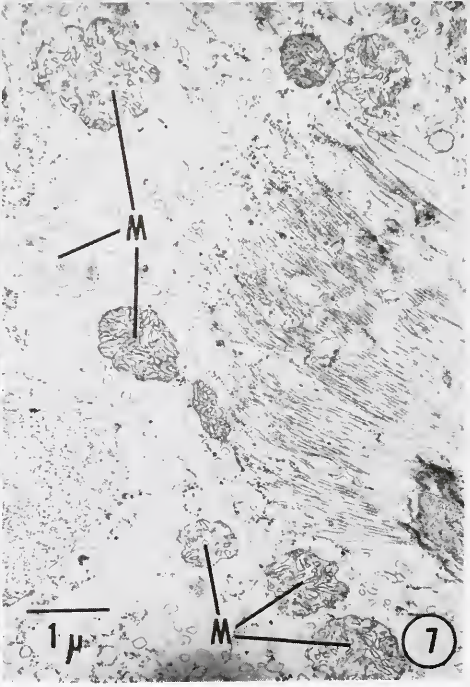
FIGURE 4. A transverse section through a normal "Fibrillenstrucktur" muscle cell. The numerous bundles or myofibrils (MFB) are separated from each other by the extensive sarcoplasmic reticulum (SR). At the edge of the cell two mitochondria (M) can also be observed.



5



6



7

TABLE II

Daphnia pulex *immobilization numbers for 23-hour observations experiment of 0–2 hour animals exposed to 10 ppm 3-amino-1,2,4-triazole at 24°C. Total number of animals exposed = 81.*

Age ± 1 hour	Length of exposure	Total number immobile	Per cent immobile
18	17	0	0
19	18	12	14.8
19.5	18.5	15	18.5
20	19	28	34.6
20.5	19.5	45	55.5
21	20	61	75.3
21.5	20.5	69	85.2
22	21	78	96.3
22.5	21.5	81	100

ment was observed. The animals sank to the bottom of the vials and often rested on their sides. In 0–16 hr old animals this was consistently between 15 and 22 hr after continuous exposure. In the vast majority of cases when an individual was found to be immobile an exuvium was also seen in the vial.

To investigate the relationships between length of exposure and time of immobility specimens of *D. pulex* were exposed continuously to 3-amino-1,2,4-triazole. Animals 0–2 hr old were individually exposed (10 ppm at 24° C) and the time and number of immobile daphnids noted (Table II). No animals tested became immobile prior to 17 hr of exposure (18 ± 1 hr of age) and all individuals tested were immobile prior to 21.5 hr of exposure (22.5 ± hr of age). Thus none of the 81 animals tested became immobile prior to the projected mean first molt time for *D. pulex* at 24° C (16.73 hr. of age) based on the temperature development time curve (Bunting, 1973) for this species. A least squares linear regression of age (Y) versus % immobile (X) gives the equation $Y = 18.41 + (0.038)X$, and calculated mean immobilization time (IT 50) of 20.32 hr with a 0.86 hr standard deviation. None of the control animals became immobile. These data suggest that the time of immobility is closely related to molt. It also suggests a delay in time of ecdysis.

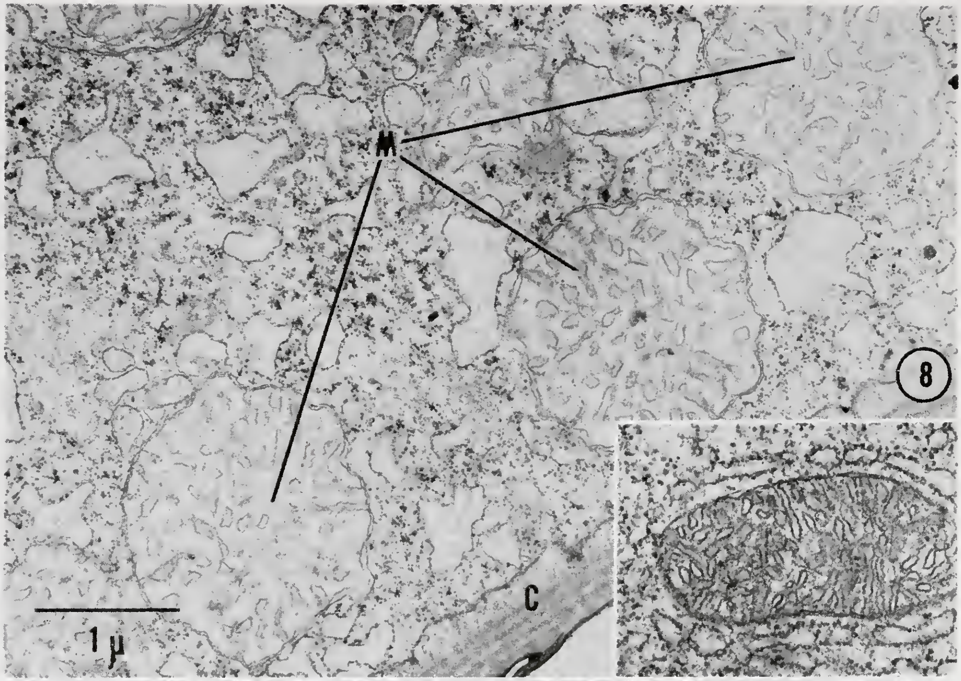
Since no immobility occurred until 18 hr of age in animals exposed to herbicide continuously after release from the brood pouch (Table II), the effect of exposure at different ages was examined. Zero to two hr old specimens of *Daphnia* were collected. Some were exposed immediately to a 3 amino-1,2,4-triazole (10 ppm at 24° C) while others were allowed to grow for intervals of 4, 8, 12, 16, 18, 20 or 24 hr before being placed in the test solution. All animals were monitored at one

---

FIGURE 5. A longitudinal section through a contracted muscle cell of an animal immobilized by 3-amino-1,2,4,-triazole. The mitochondria (M) have fewer cristae than in mitochondria of control daphnids and the matrix is less electron dense. The outer membrane of the mitochondrion is often folded (arrows). The myofibrils (MFB) are normal in appearance.

FIGURE 6. A section through the peripheral region of a muscle cell of a *Daphnia* immobilized by 3-amino-1,2,4,-triazole. More extensive damage to the mitochondria (M) was often observed. The number of cristae and electron density of the matrix are drastically reduced with folding (arrows) of the outer membrane.

FIGURE 7. A section through a muscle cell of a daphnid immobilized by 3-amino-1,2,4,-triazole. Note the variation in damage to the mitochondria (M) within a single cell.



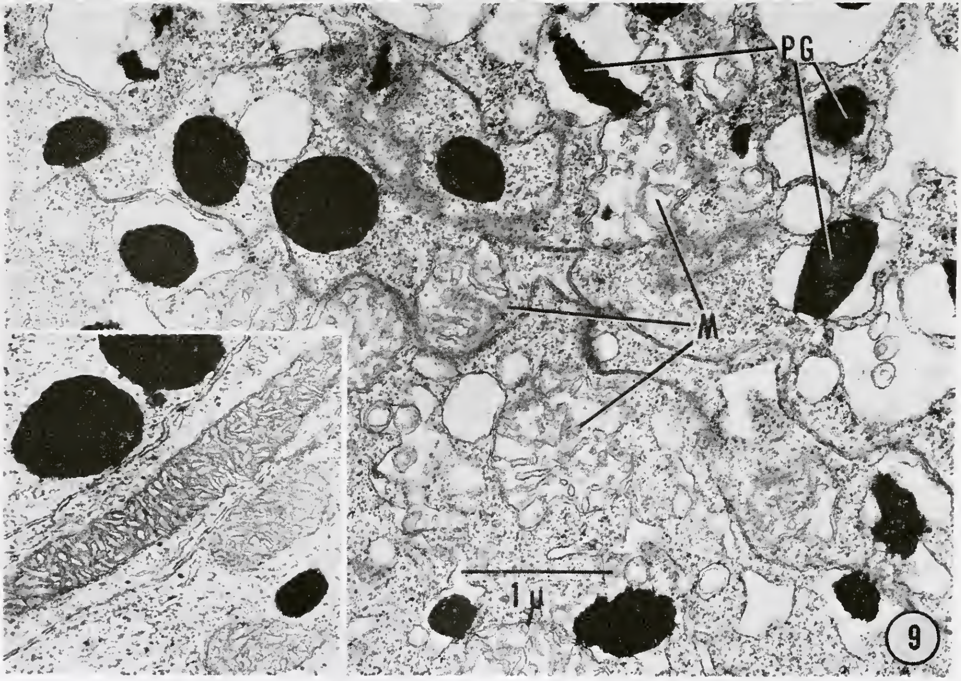


FIGURE 8. A section through the integument of an organism immobilized by 3-amino-1,2,4-triazole. The chitinous intima (c) is not altered. The mitochondria (M) of the cellular

hr intervals until immobile. Parallel controls were run and all experimental samples corrected as indicated previously. The length of time required for 95% immobility of the test animals (Y) versus the age of the animal at the time of exposure (X) was plotted (Fig. 12).

The length of exposure required to obtain immobility is inversely related to age up to 16 hr post-brood pouch release. Animals 0–2 hr old (1 ± 1 hr) required 21 hr exposure to herbicide to produce 95% immobility while animals 16 hr old (±1 hr) when placed in the herbicide became immobile after only 6 hr exposure. Thus, as daphnids approached molt, the length of exposure required to immobilize them was reduced indicating an increased sensitivity to the herbicide. Animals placed in herbicide at 18 hr of age show a reduced sensitivity as indicated by the increase in exposure time over 16 hr animals for the same degree of immobility to be produced.

If 18 hr old specimens of *Daphnia* are post-molt animals as suggested by the data of Bunting (1973), then it would appear that sensitivity to the herbicide is directly related to the events of premolt. This is further supported by the effects of herbicide on 20 and 24 hr old animals. These two groups show a reduction in time required for 95% immobilization, which is equivalent to the time they have progressed in their growth period toward the second molt.

The effect of temperature on mobility of 0–24 hr old *D. pulex* exposed to 3-amino-1,2,4-triazole was examined. Animals were exposed to the herbicide (10 ppm) for a 24 hour period at 12, 16 and 24° C. At the end of this period, the percentage immobility was calculated for each temperature. A least squares linear regression of corrected percentage immobility (Y) versus log temperature centigrade (X) was plotted (Fig. 13). The regression coefficient of the calculated line $Y = 254 + (242)X$ was found by a one-way analysis of variance to be significantly different from zero at the 0.05 level. Animals subjected to the lower temperatures were less affected by the 3-amino-1,2,4-triazole as indicated by a mean percentage immobile of 6.35 and 39.80 for 12 and 16° C, respectively, as opposed to a mean immobility for 24° C of 79.65 %. Those replications expressing zero percentage immobile after the initial 24 hour exposure at 12° C were monitored for a second day with identical results of zero percentage immobility being observed. Thus, temperature affects the percentage mobility of 0–24 hour *Daphnia* exposed to 10 ppm 3-amino-1,2,4-triazole for 24 hr.

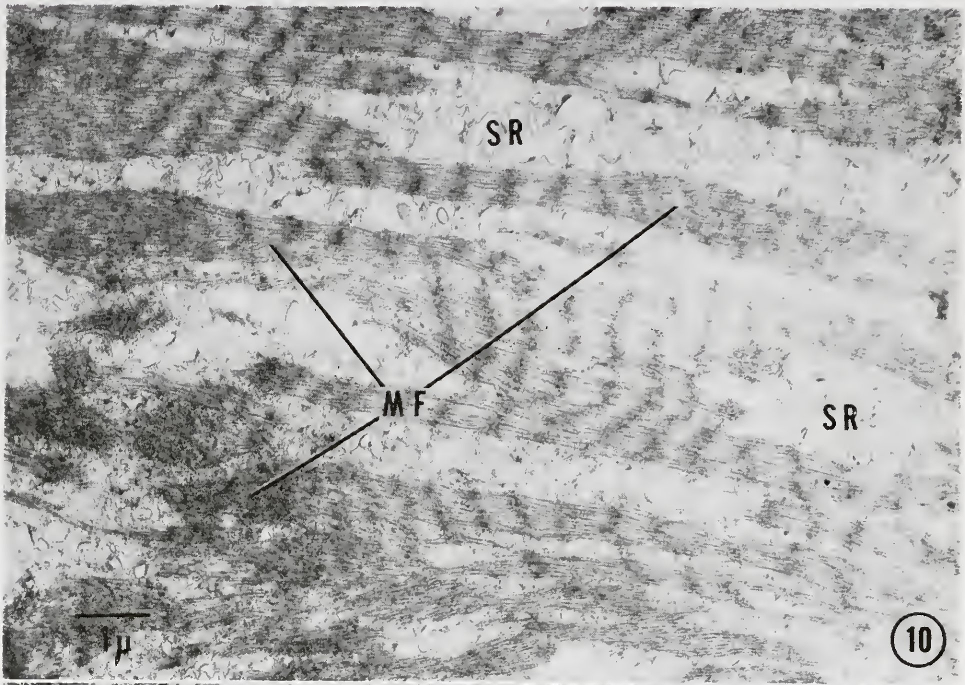
DISCUSSION

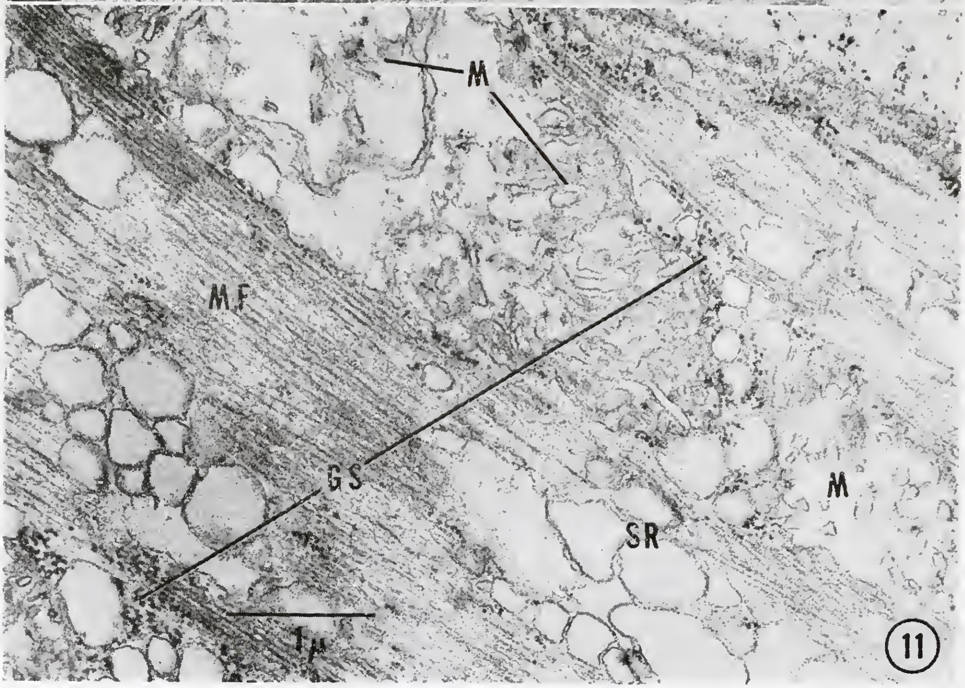
Immobility of juvenile (0–24 hr) *D. pulex* exposed to 3-amino-1,2,4-triazole appears to be tied to the molt cycle. As test animals approach ecdysis, which based on the temperature-development time curve of Bunting (1973) is 16.73 hr

---

hypodermis are swollen. This is accompanied by a reduction in electron density of the matrix and number of cristae. The insert shows a mitochondrion of a normal hypodermal cell at a comparable magnification.

FIGURE 9. A section through a receptor cell from the median compound eye of an animal immobilized by 3-amino-1,2,4,-triazole. The mitochondria (M) have fewer cristae with less electron dense matrix and show a concomitant swelling. The pigment granules (PG) are separated from their encapsulating membrane. The insert shows pigment granules and mitochondrion of a normal receptor cell at a comparable magnification.


SR
MF
SR
1μ
10


M
MF
GS
M
SR
1μ
11

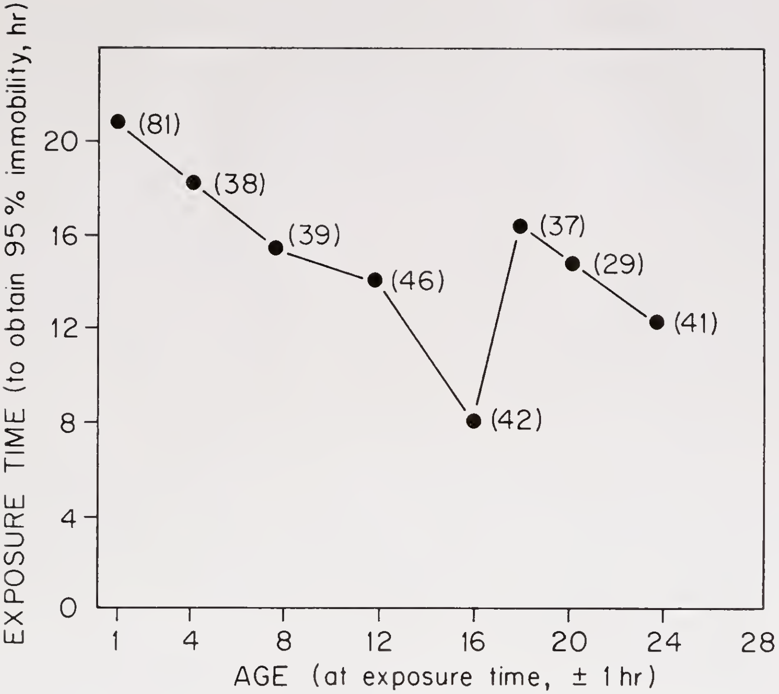


FIGURE 12. Effect of age at time of exposure to 10 ppm 3-amino-1,2,4,-triazole on the length of exposure required to obtain 95% immobility of 0-2 hr old *Daphnia pulex*. Number in parenthesis equals number of animals.

of age at 24° C, the length of exposure required for immobility decreases. However, once ecdysis is passed the required exposure time increases only to again decrease as the animals near their second molt. This is corroborated by the 6–15 hr sequential ultrastructure studies which showed no detectable organelle damage, including mitochondria, in first juvenile instar (0–24 hr old) daphnids exposed to the herbicide for up to 15 hr. Furthermore, the physiological state of intermolt adult *D. pulex* is not altered by exposure to 3-amino-1,2,4-triazole (10 ppm at 24° C) for 24 hr as indicated by the lack of alteration in the rate of oxygen consumption (Schultz, unpublished data).

Reduced temperature causes a reduced rate at which the herbicide acts on *Daphnia*. This is indicated by the fact that only 4 out of 51 test organisms were immobile after 24 hr exposure at 12° C, while at 16° C, 27 out of 70 animals tested became immobile. This is in agreement with the studies of Bunting and Robert-

---

FIGURE 10. A longitudinal section through a muscle cell of an animal immobilized by 3-amino-1,2,4,-triazole. The myofilaments (MF) show varying degrees of disorientation as the banding pattern is lost. The sarcoplasmic reticulum (SR) is swollen.

FIGURE 11. An oblique section through a muscle cell of a daphnid immobilized by 3-amino-1,2,4,-triazole. The mitochondria (M) are damaged, some to the point of being lysed. The myofilament (MF) are disarranged and the sarcoplasmic reticulum (SR) is swollen. Note the interfibrilar glycogen stores (GS).

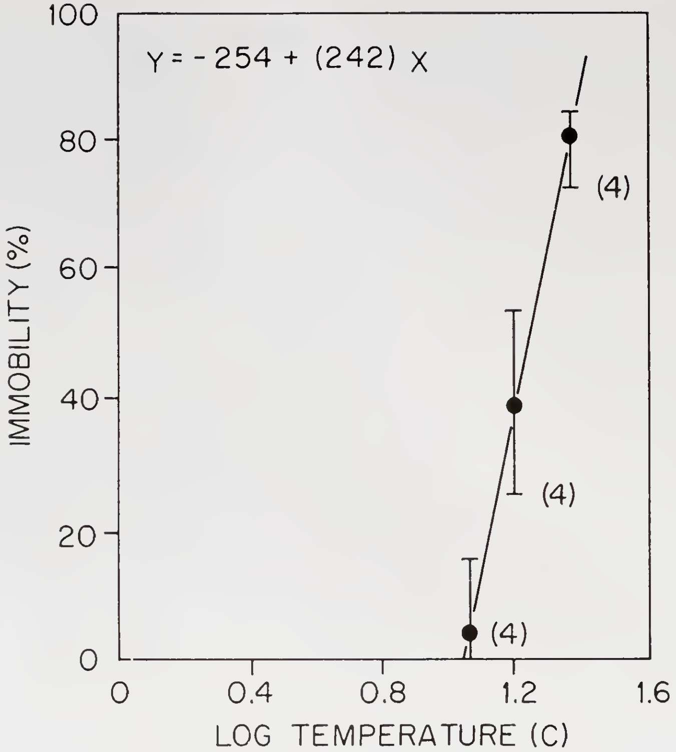


FIGURE 13. Effects of temperature on mobility of 0-24 hr old *Daphnia pulex* exposed to 10 ppm 3-amino-1,2,4-triazole. A least squares linear regression of corrected % immobility (Y) versus log temperature centrigrade (X). Number in parenthesis equals number of replications. Vertical bars indicate the range.

son (1975) who found that, in order to obtain similar rates of immobility at 15 and 20° C, it was necessary to double the amount of herbicide at the lower temperature. This may be correlated with a reduction in metabolic rate and longer instar duration at lowered temperatures, since Robertson (1971) reported that the mean first pre-adult instar duration of approximately 50 hr at 15° C was extended to 100 hr when the temperature was reduced to 10° C. Thus, at 12° C no animals would be expected to reach ecdysis (in fact even after 48 hr exposure), while at 16° C those animals which became immobile could represent the number reaching ecdysis. The time lag between projected time of ecdysis and time of immobility may be explained by a change in metabolic rate suggested by behavior changes which more closely approximate ecdysis.

The effect of 3-amino-1,2,4-triazole on plants has been extensively studied. It is readily absorbed through the foliage or roots and translocated throughout the plant (Ashton and Crafts, 1973). It causes chlorosis and inhibits regrowth from buds (Ashton and Crafts, 1973; for more in depth study see Hall, Johnson and Leinweber, 1954). Chlorosis is apparently due to the triazole interfering with light-induced changes in the proplastid resulting in a reduction in chlorophyll synthesis (Mason, 1969). Mason (1969) further notes that amitrole affects protein and purine metabolism, flavin synthesis and acts as an enzyme inhibitor.

The most striking phyotoxic symptom of 3-amino-1,2,4-triazole exposure is albinism caused by chlorophyll destruction and impairment of chlorophyll synthesis. The herbicide also blocks light-induced plastid development in wheat seedlings (Bartels, 1965). Treated plastids lacked normal granafret membrane systems as well as chloroplast ribosomes (Bartels and Weier, 1969). All of these effects take a relatively long time to culminate and either do not apply to, or can not explain the sudden immobilization observed in *Daphnia.*

However, in addition to imidazoleglycerol phosphate dehydrase inhibition in protein metabolism, 3-amino-1,2,4-triazole has been found to block several other enzyme reactions (Hein, Appleman and Pyforna, 1956; Margoliash and Novogrodsky, 1958). This, plus catalase and fatty acid peroxidase irreversible inhibition (Castelfranco and Brown, 1963), has led to the suggestion that the herbicide undergoes a one-electron oxidation to the free radical which then attacks various enzymes, causing irreversible inhibition (Castelfranco and Brown, 1963). If such were the case, it may explain the effects expressed in *Daphnia* mitochondria, since these organelles appear to be rather sensitive sites for a variety of toxic agents. For example, Kennedy and Elliott (1970) observed that tobacco cigarette smoke caused disruption of mitochondria in *Tetrahymena pyriformis.* Gray and Kennedy (1974) reported that nontobacco cigarette smoke had a similar effect on *T. pyriformis* mitochondria. They extended their studies to show that cellular respiration was also effected. Other pollutants particularly $HgCl_3$ (Grityka and Trump, 1968; Tingle, Pavlat and Cameron, 1973) have also been reported to cause gross swelling of mitochondria, decreased density of the ground matrix and deterioration of cristae. Fox and Penner (1965) in studying the effect of 3-amino-1,2,4-triazole on inhibition of tricarboxylic acid cycle substrate oxidation by isolated cucumber mitochondria noted that concentrations of 10^{-5} to 10^{-3} reduced succinate utilization in a stepwise manner but failed to affect α-ketoglutarate utilization appreciably. Lotlikar, Remmert and Freed (1968), stated that amino-triazole in concentrations as high as 10^{-2} M had less than a 5% inhibition on oxygen uptake in cabbage mitochondria when tested for 90 minutes at 30° C. Thus, the similarities of function between mitochondria and chloroplasts and their general sensitivities to 3-amino-1,2,4-triazole is not surprising. The major variation seems primarily to be associated with their different rates of action.

The overall problem in *Daphnia* appears to be one of permeability. As long as the animal is in intermolt state the rate of entry of the herbicide from a 10 ppm solution may be slow enough that the 3-amino-1,2,4-triazole may be detoxified or eliminated without expressing any physiological or cytotoxic effects. However, during the stress of ecdysis with volume increase produced by uptake of water into the cells and the change in the permeability of the body surface (Lockwood, 1967), in the influx of 3-amino-1,2,4-triazole is probably increased. Since molting in *Daphnia* is considered to be a continuous process (Procella, Rixford and Slater, 1969), it is difficult if not impossible to pinpoint the time of permeability change associated with ecdysis. This does not exclude uptake of the herbicide by the gut *via* oral and anal drinking (Fox, 1952), but since the digestive tract shows minimal damage in experimental animals this mode of entry seems unlikely. In any case, once in the animal, 3-amino-1,2,4-triazole probably inhibits enzyme reactions possibly including specific ones involved in oxidative phosphorylation thus stopping ATP synthesis and causing mitochondrial swelling. Such actions affect the most active tissue or enzyme system first, thus accounting for the consistent mitochondrial damage observed in muscle cells, the observed decreased rate and efficiency of antennal strokes and the sinking to the bottom of the vial expressed by treated animals just prior to immobilization. Disorientation of myofibrils, swelling of the sarcoplasmic reticulum, and other cell alterations are probably secondary effects caused by the breakdown of the osmoregulatory mechanism due to the lack of an external energy source, ATP. This theory is supported by the facts that the onset of immobility is: correlated with ecdysis; affected by age at time of exposure; and reduced with a reduction in temperature. It also explains the specificity towards mitochondrial damage in high energy requiring cells and the variation in other cell and tissue alteration observed in thin section.

This work was supported in part by a Sigma Xi research grant. Dr. Dewey L. Bunting assisted in the statistical analysis of the data.

SUMMARY

Zero to 24 hour old specimens of *Daphnia* were exposed to 10 ppm 3-amino-1,2,4-triazole at 24° C until immobilized. The most consistent alteration in cell structure was expressed by the mitochondria especially those of muscle fibers. Not all muscle cells within a single animal nor all mitochondria within a single cell were affected equally. The most common alteration observed was folding of the outer membrane. This was accompanied by organelle swelling and a reduction in electron density of the ground matrix and number of cristae.

In addition other cytotoxic effects were observed. These included general tissue swelling, disarrangement of myofilaments and dissociation of membranes. Mobile 0–24 hr old daphnids which were exposed to the herbicide for up to 15 hr showed no cell or organelle damage regardless of length of exposure.

Data from acute static experiments suggest that the time of immobility is closely related to molt for 0–24 hr juveniles.

LITERATURE CITED

ASHTON, F. M. AND A. S. CRAFTS, 1973. *Mode of action of herbicides.* Wiley-Interscience, New York, 504 pp.

BARTELS, P. G., 1965. Effect of amitrole on the ultrastructure of plastids in seedlings. *Plant Cell Physiol.*, **6**: 227–270.

BARTELS, P. G. AND T. E. WEIER, 1969. The effect of 3-amino-1,2,4-triazole on the ultrastructure of plastids of *Triticum vulgare* seedlings. *Amer. J. Bot.*, **56**: 1–7.

BINDER, G., 1931. Über das Muskelsystem von *Daphnia magna. Int. Rev. Hydrobiol. Hydrog.*, **26**: 54–111.

BOUGLIGAND, Y., 1962. Les ultrastructures du muscle strié, et de ses attaches au esquelete chez le cyclops (Crustaces, Copepodes). *J. Microsc.*, **1**: 377–394.

BREUKELMAN, J., 1932. Effects of age and sex on resistance of daphnids to mercuric chloride. *Science*, **76**: 302.

BROOKS, J. L., 1957. The systematics of North American *Daphnia. Mem. Conn. Acad. Arts Sci.*, **13**: 1–180.

BUNTING, D. L., 1973. Zooplankton: thermal regulation and stress. Pages 50–55, in B. J. Gallagher, Ed., *Proceedings of a symposium on "Energy production and thermal effects."* Limnetics Incorporated, Milwaukee, Wisconsin,

BUNTING, D. L. AND E. G. ROBERTSON, 1975. *Lethal and sublethal effects of herbicides on* *zooplankton species. Research Report No. 43, Water Resources Research Center,* The University of Tennessee, Knoxville, Tennessee, 35 pp.

Castelfranco, P. and M. S. Brown, 1963. A hypoothesis of amitrole action based on its behavior towards free radical generating systems. *Weeds,* **11**: 116–124.

Cowell, B. C. 1965. Effects of sodium arsenite and silver on the plankton population in farm ponds. *Trans. Amer. Fish. Soc.,* **94**: 371–377.

Crosby, D. G. and R. K. Tucker, 1966. Toxicity of aquatic herbicides to *Daphnia magna. Science,* **154**: 289-291.

Dewey, J. E. and B. C. Parker, 1964. Mass rearing of *Daphnia magna* for insecticide bioassay. *J. Econ. Entomol.,* **57**: 821–825.

Findley, D. I., 1969. Effects of amino triazole weedkiller on selected aspects of the life history of *Daphnia magna. Ph.D. dissertation, The University of Tennessee,* Knoxville, Tennessee, 62 pp. (*Diss. Abstr.,* 31/04B: 2365; order number 70-17813).

Fox, H. M., 1952. Anal and oral intake of water by crustaceans. *J. Exp. Biol.,* **29**: 583–599.

Fox, C. L. and D. Penner, 1965. Effect of inhibitors and herbicides on tricarboxylic acid cycle substrate oxidation by isolated cucumber mitochondria. *Weeds,* **13**: 226–231.

Gray, J. P. and J. R. Kennedy, 1974. Ultrastructure and physiological effects of nontobacco cigarettes on *Tetrahymena. Arch. Environ. Health,* **28**: 283–291.

Grityka, T. L. and B. F. Trump, 1968. Renal tubular lesions caused by mercuric chloride. *Amer. J. Pathol.,* **52**: 1225–1278.

Hall, W. C., S. P. Johnson, and C. L. Leinweber, 1954. Amino triazole a new abscission chemical growth inhibitor. *Tex. Agr. Exp. Sta. Bull.,* **789**: 1015.

Heim, W. G., D. Appleman, and H. T. Pyforna, 1956. Effects of 3-amino-1,2,4-triazole (AT) on catalase and other compounds. *Amer. J. Physiol.,* **186**: 19–23.

Hoyle, G., 1967. Diversity of striated muscle. *Amer. Zool.,* **7**: 435–449.

Kennedy, J. R. and A. M. Elliott, 1970. Cigarette smoke: the effect of residue on mitochondrial structure. *Science,* **168**: 1097–1098.

Lockwood, A. M. P., 1967. *Aspects of the physiology of crustacea.* W. H. Freeman and Co., San Francisco, 328 pp.

Lotlikar, P. D., L. F. Remmert, and V. H. Freed, 1968. Effects of 2,4-D and other herbicides on oxidative phosphorylation in mitochondria from cabbage. *Weed Sci.,* **16**: 161–165.

Lowndes, A. G.., 1952. Hydrogen in concentration and the distribution of freshwater Entomostraca. *Ann. Mag. Natur. Hist.,* **5**: 58–65.

Margoliash, E. and A. Novogrodsky, 1958. A study of the inhibition of catalase by 3-amino-1,2,4-triazole. *J. Biochem.,* **68**: 468–475.

Mason, C. C., 1969. Amitrole. Pages 187–206 in P. C. Kenney and D. D. Kaufman, Eds., *Degradation of herbicides.* M. Dekker Co., New York.

Meyer, F. A., 1966. Aquatic plant control. Pages 70–95 in A. Calhoun, Ed., *Inland fisheries management.* Department of Fish and Game, State of California.

Porcella, D. B., C. E. Rixford, and J. V. Slater, 1969. Molting and calcification in *Daphnia magna. Physiol. Zool.,* **42**: 148–159.

Robertson, S. D., 1971. The effects of temperature on instar duration and egg development of *Daphnia pulex* Leydig. *M.S. Thesis, The University of Tennessee,* Knoxville, Tennessee, 55 pp.

Sanders, H. O. and O. B. Cope, 1966. Toxicities of several pesticides to two species of cladocerans. *Trans. Amer. Fish Soc.,* **95**: 165–169.

Starr, R. C., 1964. The culture collection of algae at Indiana University. *Amer. J. Bot.,* **51**: 1013–1044.

Tingle, L. E., W. A. Pavlat, and I. L. Cameron, 1973. Sublethal cytotoxic effects of mercuric chloride on the ciliate *Tetrahymena pyriformis. J. Protozool.,* **20**: 301–304.

Venable, J. H. and R. Coggeshall, 1965. A simplified lead citrate stain for use in electron microscopy. *J. Cell Biol.,* **25**: 407–408.

Walker, C. R., 1964. Simazine and other S-triazine compounds as aquatic herbicides in fish habitats. *Weed,* **12**: 134–139.

Walker, C. R., 1965. Diuron, fenuron, monuron, neburon, and TCA mixtures as aquatic herbicides in fish habitats. *Weed,* **13**: 297–301.

Watson, M. L., 1958. Staining of tissue sections for electron microscopy with heavy metals. *J. Biophys. Biochem. Cytol.,* **4**: 475–478.

Reference *Biol. Bull.*, **151**: 386–397. (October, 1976)

THE CHEMICAL ECOLOGY OF *BIOMPHALARIA GLABRATA*: THE EFFECTS OF AMMONIA ON THE GROWTH RATE OF JUVENILE SNAILS

J. D. THOMAS, M. POWLES AND R. LODGE

School of Biological Sciences, University of Sussex, Falmer, Brighton
BN1 9QH, Sussex, England, U. K.

The growth and natality rates of *Biomphalaria glabrata* (Say) may be enhanced, in closed systems, by decreasing the volume or by increasing snail numbers or conditioning time to optimum thresholds. Further increases in snail numbers or a decrease in the volume per snail or in conditioning time may result in a decrease in growth and natality rates of the snail (Thomas, 1973). The environmental factors which may be involved in causing the negative feedback effects have been discussed by Thomas (1973) and include ammonia which is the main excretory product of these snails. It is well known that ammonia may be toxic to certain aquatic animals (Kawomoto, 1961; Ball, 1967; Spotte, 1970). The purpose of the work described in the present paper is to determine the extent to which ammonia can influence the growth and survival rates of the snails and thus provide the basis for a negative feedback mechanism.

Materials and Methods

The methods used for maintaining the stock cultures of the Venezuelan, albino strain of *B. glabrata* used in these experiments have been described by Thomas and Benjamin (1974). At the commencement of the experiment they had an initial weight of approximately 20 mg. They were maintained at a temperature of 26 ± 1° C with a photoperiod of 12 L: 12 D in an environmental unit described by Thomas and Benjamin (1974). They were fed on standard daily rations of washed lettuce discs or 3% Bemax made up in agar, provided in excess of requirements. Uneaten remains were removed each day. Two kinds of media made up from deionized water were used in the experiments: standard snail water [SSW(1)], with the following composition in millimoles per liter, Ca 2.0, Mg 0.13, Na 0.63, K 0.086, Cl 0.63, HCO_3 4.037, SO_4 0.13, NO_3 0.049; and SSW(2), -Ca 2.0, Mg 0.13, Na 0.63, K 0.086, Cl 4.0, HCO_3 0.67, SO_4 0.13 and NO_3 0.049.

In most of the experiments ammonia was added in the form of NH_4Cl to give concentrations ranging from 1 to 75 or 100 μg/ml NH_3 in 50 ml of SSW(1) or (2). Media without ammonia served as controls. 1.0 μg/ml of ammonia was used as a unit because it was estimated to be the mean concentration of ammonia produced in one day by 20 mg snails kept at a density of one snail per 50 ml (Powles, unpublished). Ammonia was also supplied as $(NH_4)_2SO_4$ in some of the experiments in order to ascertain whether the effects observed were similar to those produced by NH_4Cl.

As the relative amounts of ionized and free ammonia are dependent on pH, the effects of varying ammonia concentrations were investigated in media with the

TABLE I

The mean cumulative specific growth rates ($\bar{x}$) *achieved by juvenile* B. glabrata *fed on Bemax and subjected to various concentrations of ammonia (as* NH_4Cl*) in SSW(1) buffered at pH 7 and 9 with Tris and Borate buffers respectively. (N = number of snails, s.e. = standard error, *, **, *** differ from controls at* $P < 0.05, 0.01, 0.001$ *respectively).*

Buffer used	Duration of experiment in days	Concentration of ammonia in µg/ml																	
		0			1			10			25			50			75		
		N	x̄	s.e.	N	x̄	s.e.	N	x̄	s.e.	N	x̄	s.e.	N	x̄	s.e.	N	x̄	s.e.
Tris	0–3	10	0.67	± 0.01	10	4.33*	± 1.67	10	1.0	± 0.33	10	1.33	± 0.33	10	2.33	± 1.00	10	3.00	± 2.00
Tris	0–6	10	2.67	± 0.01	10	6.33*	± 1.33	10	6.5*	± 1.00	10	5.50	± 1.50	10	7.00*	± 1.33	10	6.17	± 2.00
Tris	0–9	10	2.89	± 0.56	10	6.33*	± 1.11	10	6.0**	± 0.89	10	6.22	± 1.67	10	7.56***	± 1.00	10	6.22	± 1.78
Borate	0–3	10	2.33	± 0.57	10	4.00	± 1.0	6	3.33	± 1.33	1								
Borate	0–6	9	3.50	± 0.89	9	3.00	± 1.17	5	2.17	± 0.83	1								
Borate	0–9	0	—		0	—		0	—		0								

following pH values: 5.4–5.5, 7.0, 8.1–8.6, and 9.0. The pH values of 5.4–5.5 and 8.1–8.6 were those of SSW(2) and SSW(1), respectively. Tris and borate buffers were used to achieve pH values of 7 and 9.0 respectively (Dawson, Elliot, Elliott and Jones, 1969). Various other buffers including citrate and glycine buffers were tried and rejected for various reasons including the fact that they formed precipitates on standing in SSW. Both of the buffers used in the present experiments have also been used successfully as buffers in perfusion fluids for invertebrates (Welsh and Smith, 1960).

Assay snails were kept in isolation in 50 ml of nonaerated test or control media. Each treatment was replicated ten times. The experiments which were based on a completely randomized design were subjected to analysis of variance.

Growth was monitored at three day intervals using a weighing method described by Thomas and Benjamin (1974). The growth rate was expressed as the cumulative specific growth rate (Wt-Wo) × 100/Wo × Δt, where Wo represents weight in mg at time t and Δt is time interval in days.

RESULTS

With the exception of the experimental snails kept in borate buffer SSW(1), the various treatments did not affect the survival rates over the period of the experiment. Table I gives the number of snails placed in borate or tris buffered media surviving to be weighed at the end of each three day interval. As ten snails were used initially in each treatment, it is evident that heavy mortality had occurred in the borate buffered treatments. With one exception all the snails in borate buffers in the 25 and 50 μg/ml treatments had died within three days and by the end of nine days all the remainder had died. It is also evident from Table I that the growth rates of the snails in SSW buffered by both tris and borate had been seriously impaired, as it was only about 10 per cent of that achieved by the control snails in unbuffered SSW (Table II).

It can be seen from Tables I and II, which give the mean cumulative growth rates achieved by the snails in the various treatments that the values for the control snails tend to be rather variable. Factors which influence the growth rate of the snails include their physiological state or growth potential as well as their age and the nature of the medium and the food. It has been found that standardization of the methods of maintaining stock cultures, by using a flow-through system, and of handling procedures has helped to reduce the variability in the growth rates of the snails. Although lettuce is a good quality food which allowed the snails to grow rapidly, it is evident from Table I and II that the standard errors of the mean for lettuce-fed snails tend to be higher than those for snails fed on Bemax. The latter was, therefore, adopted as a food medium in subsequent experiments. The standard errors also tend to increase as the snails become larger. To overcome these problems and to facilitate comparisons between experiments, the mean specific growth rates have been expressed as percentages of the mean control values (Figs. 1–3). Tables I and II show that statistically significant differences between the controls and certain of the treatments may occur as early as the third day. It might be expected that the number of statistically significant treatment effects would increase with time, but this is not necessarily the case in all experiments. In general, however, there is a measure of consistency and the same treatments tend to show statistically significant effects throughout the duration of an experiment.

TABLE II

Mean cumulative specific daily growth rates ($\bar{x}$) achieved by juvenile Biomphalaria glabrata *in the two media SSW(1) and SSW(2) when the concentrations of ammonia were varied from 0–100 μg/ml, P = results of analysis of variance, (*, **, *** differ from controls at $P < 0.05, 0.01, 0.001$ respectively).*

Treatments			Concentrations of NH_3 in μg/ml								P values
Medium	Food source	NH_3 source	Days	0 $\bar{x}$ ±s.e.	1 $\bar{x}$ ±s.e.	10 $\bar{x}$ ±s.e.	25 $\bar{x}$ ±s.e.	50 $\bar{x}$ ±s.e.	75 $\bar{x}$ ±s.e.	100 $\bar{x}$ ±s.e.	
SSW(1)	Lettuce	NH_4Cl	0–3	32.67 ±4.00	36.67 ±6.00	25.67 ±5.67	14.33** ±3.33	13.33** ±3.00	10.67** ±2.67	7.33 ±0.67***	<0.001
SSW(1)	Lettuce	NH_4Cl	0–6	31.67 ±3.67	35.67 ±7.83	24.33 ±4.17	17.67 ±4.67*	17.17 ±5.50*	17.17 ±3.00*	13.83 ±3.17	<0.05
SSW(1)	Lettuce	NH_4Cl	0–9	30.89 ±4.67	40.33 ±7.56	24.89 ±5.56	21.78 ±4.56	20.33 ±5.00	21.44 ±4.11	15.11 ±1.11**	<0.05
SSW(1)	Bemax	NH_4Cl	0–9	48.44 ±3.22	51.11 ±2.67	34.56 ±6.44	27.67 ±3.78***	8.33 ±3.33***	3.78 ±1.89***	0.33 ±0.11***	<0.001
SSW(1)	Bemax	$(NH_4)_2SO_4$	0–3	19.0 ±1.33	16.33 ±1.33	15.33 ±1.67	11.67 ±1.00***	8.0 ±1.00***	2.33 ±1.00***	0.10 ±0.04***	<0.001
SSW(1)	Bemax	$(NH_4)_2SO_4$	0–6	29.67 ±2.50	21.67 ±1.83*	25.33 ±2.67	22.33 ±1.33*	12.0 ±1.50***	4.0 ±1.17***	1.33 ±0.33***	<0.001
SSW(1)	Bemax	$(NH_4)_2SO_4$	0–9	43.44 ±1.89	30.22 ±1.44***	39.22 ±3.78	37.11 ±2.0*	26.67 ±3.22***	11.22 ±2.33***	6.89 ±2.23***	<0.001
SSW(2)	Bemax	NH_4Cl	0–3	24.33 ±1.67	18.67 ±0.67**	13.67 ±1.33***	13.33 ±1.67***	12.33 ±1.33***	9.00 ±0.03***	12.33 ±0.15***	<0.001
SSW(2)	Bemax	NH_4Cl	0–6	24.50 ±2.83	22.00 ±1.33	20.50 ±1.33	21.00 ±1.50	16.67 ±1.50*	15.83 ±1.83*	5.33 ±1.33***	<0.01
SSW(2)	Bemax	NH_4Cl	0–9	32.22 ±3.33	31.11 ±2.67	31.33 ±3.44	19.67 ±1.78**	14.67 ±1.33***	12.11 ±1.56***	20.11 ±2.56**	<0.01
SSW(2)	Bemax	NH_4Cl	0–3	1.33 ±0.33	4.67 ±1.33*	2.33 ±1.00	4.67 ±1.00**	2.67 ±0.67	1.0 ±0.33	1.33 ±0.67	<0.001
SSW(2)	Bemax	NH_4Cl	0–6	9.00 ±0.83	13.00 ±2.50	7.83 ±1.33	11.17 ±1.83	7.17 ±0.50	7.00 ±1.50	3.17 ±1.00***	<0.01
SSW(2)	Bemax	NH_4Cl	0–9	15.67 ±0.89	19.78 ±0.44***	14.56 ±2.11	14.78 ±0.89	10.44 ±1.22**	12.11 ±1.33*	8.33 ±0.56***	<0.01
SSW(2)	Bemax	$(NH_4)_2SO_4$	0–3	21.00 ±1.33	20.0 ±1.67	20.00 ±1.67	23.33 ±1.67	24.67 ±1.00*	22.0 ±2.00	2.0 ±1.33***	<0.01
SSW(2)	Bemax	$(NH_4)_2SO_4$	0–6	24.83 ±2.50	21.33 ±1.33	34.67 ±3.00*	24.0 ±4.33	22.0 ±1.00	22.83 ±1.67	7.0 ±2.83***	<0.01
SSW(2)	Bemax	$(NH_4)_2SO_4$	0–9	27.56 ±2.11	34.44 ±1.67	25.44 ±1.44	22.89 ±1.00	24.00 ±1.33	14.89 ±1.78***	12.0 ±2.33***	<0.001

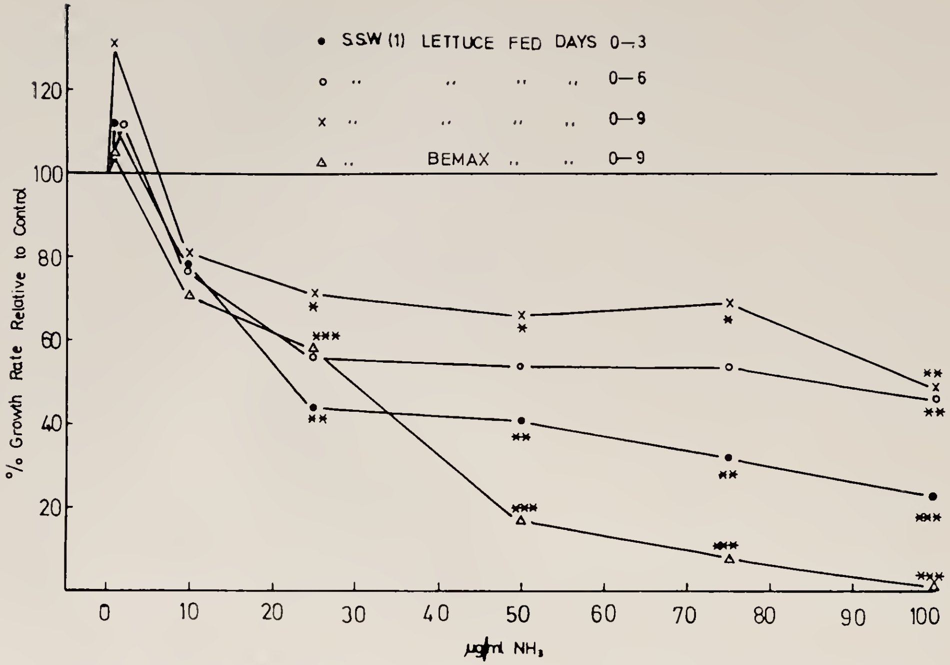


FIGURE 1. Percentage growth rate of snails relative to that of control snails when subjected to various concentrations of ammonia in SSW(1) and fed with either lettuce or bemax.

Analyses of variance undertaken on these data in Tables I and II show that all the treatments, with the exception of those involving buffered media, caused statistically significant treatment effects ($P < 0.01$–0.001). These can be attributed to the fact that the growth rates of the snails tend to be either enhanced or inhibited to a statistically significant extent compared with controls. Enhancement of growth was more commonly encountered in the treatments containing 1 μg/ml of ammonia. In six of these cases the mean cumulative specific growth rates were significantly higher than the controls; three occurred in the tris buffered treatments, two in those in which ammonia was added to SSW (2) as NH_4Cl and one in which it was added as $(NH_4)_2 SO_4$. Three examples of significant growth enhancemnet also occurred in the treatments receiving 10 μg/ml of ammonia; two occurred in the tris-buffered treatments and one in the chloride medium in which ammonia had been added as ammonium chloride. One case of significant growth enhancement also occurred in each of the treatments receiving 25 and 50 μg/ml of ammonia. It is noteworthy that most of the examples of statistically significant growth enhancement caused by the addition of ammonia occurred in experiments in which the snails were growing relatively slowly.

Growth inhibition also occurred in treatments to which ammonia had been added. The percentage of treatments showing statistically significant growth reduction increased progressively with increase in ammonia concentration above the optimum levels. Thus if the treatments were buffered with borate or tris are omitted, 18.7, 6.2, 56.2, 68.7, 68.7 and 93.7% of the treatments receiving 1, 10, 25, 50, 75 and 100 μg/ml of ammonia, respectively, show a significant growth reduction. A comparison of Figures 2 and 3 show that the percentage reductions tend to be greater in SSW(1), in which the dominant anion is bicarbonate, than in SSW(2) which has chloride as the dominant anion.

Table III shows that the initial mean pH values are considerably higher in SSW(1) than in SSW(2). However, the pH values of the media decline as a result of conditioning by the snails. It can be seen from Table III that the decrements in pH become progressively greater as the snails become larger. The values in SSW(1) always remain on the alkaline side of neutrality whereas those in SSW(2) reach values as low at 4.6. At any one time the differences between treatments are very slight, although there is some tendency for pH values in SSW(1) to decrease progressively with ammonia concentration by the sixth and ninth days.

The pH values are important because they influence the relative quantities of free ammonia as indicated by the following equation: conc of free ammonia = total ammonia conc/1 + antilog (pKa − pH). If it is assumed that the various media have the following pH values: SSW(2), 6.5; SSW(1) tris-buffered, 7.0; SSW(1), 8.3; and SSW(1) borate-buffered, 9.0 then the calculated values for percentage free ammonia are 0.17, 0.55, 9.82 and 35.46%, respectively. In consequence, the initial concentrations of free ammonia in SSW(2), SSW tris-buffered, SSW(1) and SSW(1) borate-buffered media to which ammonia has been added vary from

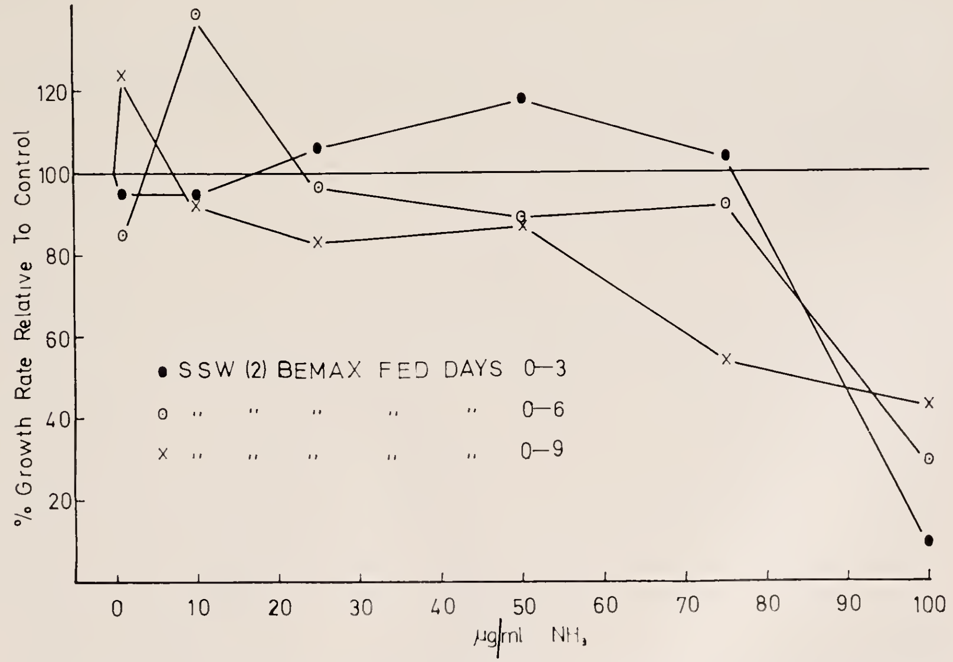


FIGURE 2. Percentage growth rate achieved by snails relative to that of control snails when subjected to various concentrations of ammonia in SSW(2) and fed with bemax.

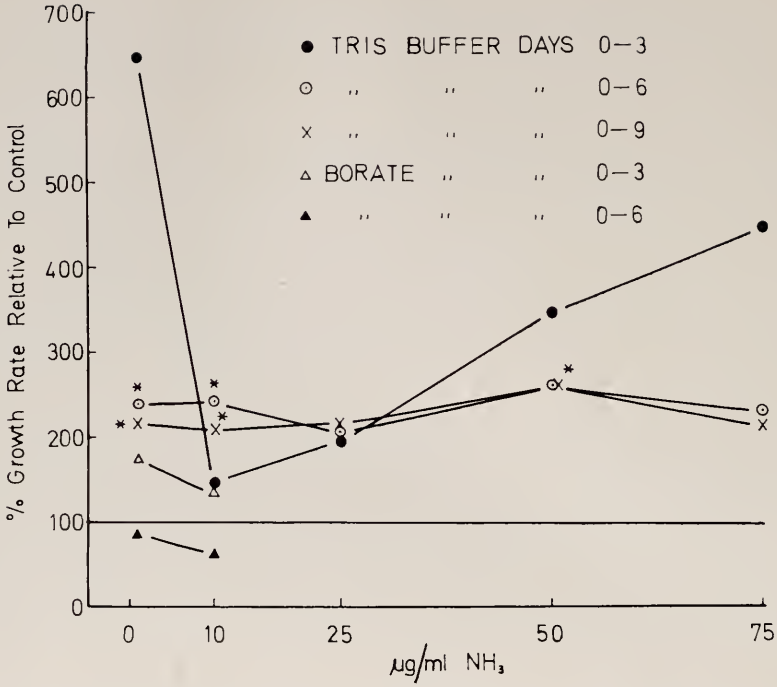


FIGURE 3. Percentage growth rate achieved by snails relative to that of control snails when subjected to various concentrations of ammonia in tris or borate buffers.

1.74×10^{-3} to 1.74×10^{-1}, 5.46×10^{-3} to 5.46×10^{-1}, 9.82×10^{-2} to 9.82 and 3.55×10^{-1} to 35.46 µg/ml respectively.

DISCUSSION

The net movement of ammonia across barriers depends on the partial pressure, P_{NH_3}, on each side of the membranes and occurs from higher to lower tensions. It

TABLE III

The mean pH values in the various treatments at different times. T_0, T_1, T_2 and T_3 correspond with the times when the snails were weighed. The values at time T_0 are therefore the initial values and those at T_1, T_2, T_3 are the values after the snails had conditioned the media for consecutive three day periods.

Medium	Time when measurements taken	Concentration (µg/ml NH_3)						
		0	1	10	25	50	75	100
SSW(1)	T_0	8.6	8.5	8.5	8.4	8.3	8.2	8.1
	T_1	8.7	8.6	8.4	8.3	8.2	8.1	8.0
	T_2	7.5	7.5	7.6	7.6	7.8	7.9	7.8
	T_3	7.2	7.2	7.3	7.5	7.6	7.9	7.8
SSW(2)	T_0	6.5	6.4	6.3	6.3	6.2	6.2	6.2
	T_1	5.5	5.5	5.6	5.5	5.4	5.4	5.4
	T_2	4.7	4.7	4.7	4.7	4.6	4.6	4.6
	T_3	4.6	4.6	4.6	4.6	4.6	4.6	4.6

has been shown that P_{NH_3} is proportional to pH: pH = pK + log NH_3/NH_4 (Maetz, 1972). Free ammonia will, therefore, pass from the side of higher to lower pH. As free ammonia diffuses readily across membranes because of its high lipid solubility and the ambient pH of 9.0 in the borate medium is probably higher than that of the internal environment, it is to be expected that outward diffusion would be prevented and inward diffusion facilitated. This hypothesis is supported by observations which indicate that the pH of the pallial fluid of molluscs varies from 7.7–8.4 (Stolkowski, 1951) and molluscan blood from 7.4–7.6 (Speeg and Campbell, 1968). The internal ammonia concentrations of snails in media buffered by borate may, therefore, rapidly reach toxic proportions even in the control treatments to which ammonia had not been added.

It has been shown that snails alter the chemical composition of the medium in which they live by taking up ions such as Na^+, Ca^{++} and Fe^{+++} and by releasing other such as NH_4^+, H^+ and HCo_3^- (Thomas, Goldsworthy and Aram, 1975; Thomas and Aram, 1974). Recent work (Maetz, 1972) has shown that the release of endogenous ions such as NH_4^+ and HCO_3^- by aquatic animals including snails may be tightly coupled with the uptake of exogenous ions such as Na^+ and Cl^-, respectively. It is possible, therefore, that the buffered media interfere with the coupled exchange of ions. An alternative explanation is that both 'tris' and borate ions are taken up by the snails and have an adverse effect on their physiology. Tris-buffered solutions may also be harmful because they absorb CO_2 from the air (Dawson *et al.*, 1969).

Since free ammonia passes from the side of higher to lower pH, (Maetz 1972) and the pH of the pallial fluid in molluscs is 7.7–8.4, (Stolkowski, 1951) and that of molluscan blood 7.4–7.6 (Speeg and Campbell, 1968), it is possible that *B. glabrata* may take up free ammonia from SSW(1) and borate-buffered media as their pH's are 8.3–8.5 and 9.0, respectively. It is unlikely, however, that free ammonia can enter the snail from the tris-buffered media and SSW(2), as the pH values are 7.0 and < 7.0, respectively. On the other hand, there is a possibility that even in these two media the snails may take up NH_4^+ from the medium by active transport (Maetz, 1972). According to Fromm and Gillette (1968), there is a direct linear correlation between the concentration of ambient ammonia and that of blood ammonia in aquatic animals, including fish, when they are exposed to varying concentrations of ammonia. The snails may, therefore, take up ammonia from the medium in both the dissociated and undissociated form.

As free ammonia is a proton acceptor ($NH_3 + H^+ = NH_4^+$), once it enters the snail it may become involved in several physiological processes which are beneficial and result in growth enhancement. First, ammonia may help to maintain the pH of the blood. Carbonic anhydrase in the mantle tissue catalyzes the production of HCO_3^- and H^+ according to the following reaction: $CO_2 + H_2O = HCO_3^- + H^+$. If the proton is then captured by NH_3, the mantle tissues may act as a diffusion trip for the blood CO_2 and help to control the CO_2 concentration and pH of the blood. The involvement of ammonia in controlling the pH of fish blood has been demonstrated experimentally by Wolbach, Heinemann and Fishman (1959).

Secondly, free ammonia may also facilitate the deposition of $CaCO_3$ in areas of shell growth. According to Berner (1968), Speeg and Campbell (1969), and Campbell and Bishop (1970), there are good reasons for believing that ammonia may function in the deposition of calcium carbonate *via* the following reaction:

$NH_3 + HCO_3^- + Ca^+ = CaCO_3 + NH_4^+$. This has recently been tested as a model for the geochemical deposition of $CaCO_3$ (Berner, 1968).

Other mechanisms that may be facilitated by ammonia include arginine metabolism (Castaneda, Martuscelli and Mora, 1967), and the uptake of ions. It has been suggested by Maetz (1972) that NH_4^+ may be involved in the uptake of Na^+ from the medium by means of a tightly coupled exchange mechanism.

In view of the potential importance of ammonia to snails, it has been suggested by Speeg and Campbell (1969) that they may be able to control its release by synthesizing urea which is then broken down by urease. This enzyme has been shown to occur in many snails (Florkin, 1966; Hammen, Hanlon and Lum, 1962; Hammen, Miller and Geer, 1966; Speeg and Campbell, 1969). The latter authors have also produced other evidence in support of their theory, including the apparent involvement of arginine and arginase. They argue that since urea synthesis, *de novo,* starts with NH_4^+ either directly as NH_4^+ or as a glutamine and HCO_3^- and requires an expenditure of at least 2 moles of ATP it is difficult to envisage it simply as an excretory mechanism. Purine and purine nucleoside deaminases are also present in molluscs (Campbell, Drotman, McDonald and Tramell, 1972). However, because *B. glabrata* is predominantly a herbivore, it can be postulated that its requirements for detoxifying excess ammonia from protein catabolism may be minimal and it is possible that purine biosynthesis may suffice (Campbell, et al, 1972). It can be suggested, therefore, that in view of the physiological importance of ammonia to the snail, it will be selectively advantageous for them to be able to take up ambient ammonia when necessary. It is possible that this source of ammonia may be particularly important to the slow growing snails.

Biomphalaria glabrata seems able to withstand higher levels of ambient ammonia than many other aquatic organisms. Kawomoto, (1961) found that 0.3 μg/ml NH_4Cl inhibited growth of carp, while Burrows (1964) claims that concentrations of NH_4OH varying from 0.3–0.7 μg/ml (0.006–0.018 μg/ml NH_3) caused hyperplasia in the gill filaments of salmon parr. According to Ball (1967) the asymptotic LC_{50} values for perch and trout were 0.29 and 0.41 μg/ml N of undissociated ammonia. In the present investigation it was found that the survival of the snails was not affected except in the media buffered by borate. The growth of the snails was not often inhibited until the total concentration of ammonia was 25 μg/ml. This is equivalent to a concentration of 2.455 μg/ml and 0.0435 μg/ml of free ammonia in the SSW(1) and SSW(2), respectively. Molluscs also generally have higher concentrations of ammonia in their blood compared with other organisms. Thus, species of *Helix, Otala, Anodonta*, and *Sepia* have 7.20, 3.6, 0.51–0.71 and 28–48 μg/ml of ammonia compared with < 1.0 μg/ml in amphibia and 0.1–0.3 μg/ml in mammals (Prosser and Brown, 1962; Speeg and Campbell, 1968).

The growth inhibitory effect may be caused in two ways. First, the ammonium ion in the external medium may compete with ions such as sodium and possibly calcium for available transport sites (Shaw, 1960; Maetz, 1972). Secondly, excess NH_3 and NH_4 may cause toxic effects at the cellular level by interfering with the process of proton translocation across mitochondrial membranes which occur during phosphorlylation (Campbell *et al.*, 1972). According to Spotte (1970) and Maetz (1972), most of the studies concerning toxicity of ammonia in ambient water indicate that the toxicity of any given ammonia solution is augmented when the external pH is elevated due to the fact that the concentration of free ammonia increases with pH. The present study provides some support for this hypothesis because the inhibitory effects have the following sequence: borate buffered medium > SSW(1), > SSW(2). Apparently, however, there are cases where toxicity of ammonia is augmented by a decrease in pH when the latter is caused by free carbon dioxide (Maetz, 1972).

One important question is whether exogenous ammonia produced by snails could provide a mechanism for controlling growth at the individual or population level of organization in closed systems, under experimental conditions. Previous investigations (Thomas, Benjamin and Lodge, unpublished) have shown that after three days of conditioning by *B. glabrata* in the 100, 300, 500 and 700 mg weight categories, kept at densities of eight snails per 200 ml of SSW(1) (25 ml per snail), the mean concentrations of ammonia were 9.3, 8.5, 13.5 and 6.8 μg/ml NH_3 respectively. The results of the present investigation indicate that these concentrations would only exert a weak inhibitory effect. The fact that the snails also release hydrogen ions into the medium during the conditioning process and thus help to detoxify the free ammonia, also militates against the hypothesis that ammonia provides the basis of a negative feedback mechanism, except possibly when snails are kept in SSW(1) or SSW(2) under very high density conditions for long periods.

The other environmental factors that may be involved in inducing the negative feedback effects observed when snails are kept under sub-optimal density or volume conditions have been discussed by Thomas (1973) and Thomas, Lough and Lodge (1975). Under certain circumstances these effects may be caused by depletion of resources including oxygen, food and ions such as calcium or iron as well as by substances released into the medium by the snails, their food supply or by microorganisms. Thomas, Lough and Lodge (1975) concluded that although the snails appeared to produce factors which enhance growth there is no evidence that they also produce specific factors, which have a detrimental effect on growth, reproduction or survival like those which Berrie and Visser (1963) and Rose and Rose (1961) claimed to have demonstrated in media containing molluscs and tadpoles respectively. Further work is required before a full understanding of the negative feedback mechanisms involved in regulating snail populations can be achieved.

In the natural environment, although ammonia is a major excretory product of other aquatic organisms as well as snails and is also a product of bacterial decomposition, its concentration tends to be low because of the rapidity with which it is oxidized by bacteria, assimilated by plants or lost by diffusion into the air. Thus, Schutte and Frank (1964) state that only very small amounts of ammonia were found occasionally in freshwater bodies in the Transvaal. This also appears to be the case in other freshwaters in Africa (Talling and Talling, 1965) and in the temperate regions of the world (Hutchinson, 1957). According to Talling and Talling (1965) the distribution of ammonia nitrogen in the surface water of African lakes is probably less than 0.040 μg/ml although much larger amounts are detectable in the deoxygenated, lower layers of stratified lakes. One of the highest concentrations of ammonia recorded by Hutchinson (1957) was 0.168–0.544 μg/ml NH_3N in Lake Manona when it was subjected to sewage contamination. It can be concluded, therefore, that except perhaps for snails living under crowded conditions in closed, alkaline waters, ammonia is unlikely to be a major factor limiting the growth of the snails in nature.

We wish to thank the Overseas Development Administration, WHO Geneva and Shell Research Ltd., for financial help and Mrs. W. Cocks and Mr. A. Lough for assistance and their interest in the work.

SUMMARY

When juvenile specimens of *Biomphalaria glabrata* were subjected to concentrations of ammonia ranging from 1–100 μg/ml in various media the following effects were observed: the addition of ammonia to borate buffered media caused mortality. Both borate and tris-buffered media caused a decrease in the growth rate of snails when compared with controls in SSW. The growth rates of the snails could be enhanced by increasing the concentration of ammonia to critical thresholds, but further increases beyond these thresholds resulted in growth inhibition. The toxicity of ammonia in ambient water was augmented by an an increase in pH. The possible causation and ecological significance of these effects are discussed. There are indications that the snails are physiologically well-adapted to utilize ammonia when required and also to control its excretion and uptake from the medium.

LITERATURE CITED

Ball, I. R., 1967. The relative susceptibilities of some species of freshwater fish to poisons: I. Ammonia. *Water Res.*, **1**: 767–775.

Berner, R. A., 1968. Calcium carbonate secretions formed by the decomposition of organic matter. *Science*, **159**: 195–197.

Berrie, A. D. and S. A. Visser, 1963. Investigations of a growth-inhibiting substance affecting a natural population of freshwater snails. *Physiol. Zool.*, **36**: 167–173.

Burrows, R. E., 1964. Effects of accumulated excretory products on hatchery reared salmonids. *U. S. Bur. Sport Fish. Wildlife Resour. Publ.*, **66**: 1–12.

Campbell, J. W. and S. H. Bishop, 1970. Nitrogen metabolism in molluscs. Pages 103–256 in J. W. Campbell, Ed., *The invertbrata: comparative biochemistry of nitrogen metabolism.* Academic Press, London and New York.

Campbell, J. W., R. D. Drotman, J. A. McDonald and P. R. Tramell, 1972. Nitrogen metabolism in terrestrial invertebrates. Pages 1–54 in J. W. Campbell and L. Goldstein, Eds., *Nitrogen metabolism and the environment.* Academic Press, London and New York.

Castaneda, M., J. Martuscelli, and J. Mora, 1967. The catabolism of *L. arginine* by *Neurospora crassa. Biochem. Biophys. Acta*, **141**: 276–286.

Dawson, R. M. C., D. C. Elliott, W. H. Elliott and K. M. Jones, 1969. *Data for biochemical research*, Clarendon Press, Oxford, 654 pp.

Florkin, M., 1966. Nitrogen metabolism. Pages 309–351 in Wilbur and Yonge, Eds., *Physiology of Mollusca II.* Academic Press, New York and London.

Fromm, P. O. and J. R. Gillette, 1968. Effect of ambient ammonia on blood ammonia and nitrogen excretion of rainbow trout (*Salmo gairdneri*). *Comp. Biochem. Physiol.*, **26**: 887–896.

Hammen, C. S., D. P. Hanlon and S. C. Lum, 1962. Oxidative metabolism of *Lingula. Comp. Biochem. Physiol.*, **5**: 185–191.

Hammen, C. S., H. F. Miller Jr. and W. M. H. Geer, 1966. Nitrogen excretion of *Crassostrea virginica. Comp. Biochem. Physiol.*, **17**: 1199–1200.

Hutchinson, G. E., 1957. *A treatise in limnology: I. Geography, physics and chemistry.* John Wiley and Sons, New York, 1015 pp.

Kawamoto, N. Y., 1961. The influence of excretory substances of fish on their own growth. *Progr. Fish Cult.*, **23**: 70–75.

MAETZ, J. 1972. Interaction of salt and ammonia transport in aquatic organisms. Pages 105–154 in J. W. Campbell and L. Goldstein, Eds., *Nitrogen metabolism and the environment.* Academic Press, London.

PROSSER, C. L. AND J. A. BROWN, 1962. *Comparative animal physiology,* Saunders Philadelphia, London, 688 pp.

ROSE, S. M. AND F. C. ROSE, 1961. Growth-controlling exudates of tadpoles. *Symp. Soc. Exp. Biol.,* **15**: 207–218.

SCHUTTE, C. H. J. AND G. H. FRANK, 1964. Observations on the distribution of freshwater molluscs and chemistry of the natural waters in the south eastern Transvaal and adjacent northern Swaziland. *Bull. World Health Organ.,* **30**: 389–400.

SHAW, J., 1960. The absorption of sodium ions by the crayfish *Astacus pallipes hereboullet III.* The effect of other cations in the external solution. *J. Exp. Biol.,* **37**: 548–556.

SPEEG, K. V. JR. AND J. W. CAMPBELL, 1968. Formation and volatilization of ammonia gas by terrestrial snails. *Amer. J. Physiol.,* **214**: 1392–1402.

SPEEG, K. V. JR. AND J. W. CAMPBELL, 1969. Arginine and urea metabolism in terrestrial snails. *Amer. J. Physiol.,* **216**: 1003–1012.

SPOTTE, S. H., 1970. *Fish and invertebrate culture.* Wiley-Interscience, New York, 145 pp.

STOLKOWSKI, J., 1951. Essai sur le determinisme des formes minerologique du calcaire chez les êtres vivants (calcaires coquilliers). *Ann. Inst. Oceanogr. Paris,* **26**: 1–113.

TALLING, J. F. AND I. B. TALLING, 1965. The chemical composition of African lakewater. *Int. Rev. Ges. Hydrobiol.,* **50**: 421–463.

THOMAS, J. D., 1973. Schistosomiasis and the control of molluscan hosts of human schistosomes with particular reference to possible self regulatory mechanisms. Pages 387–394 in B. Dawes, Ed., *Advances in parasitology, Vol. II.* Academic Press, London, New York.

THOMAS, J. D. AND R. H. ARAM, 1974. The chemical ecology of *Biomphalaria glabrata* (Say). The effects of media homotypically conditioned by adult snails on the growth of juveniles. *J. Exp. Zool.,* **190**: 329-334.

THOMAS, J. D. AND M. BENJAMIN, 1974. The effects of population density on growth and reproduction of *Biomphalaria glabrata,* (Say) (Gasteropoda: Pulmonata). *J. Anim. Ecol.,* **43**: 31–50.

THOMAS, J. D., G. J. GOLDSWORTHY AND R. H. ARAM, 1975. Studies on the chemical ecology of snails: the effects of chemical conditioning by adult snails on the growth of juvenile snails of the same species. *J. Anim. Ecol.,* **44**: 1–27.

THOMAS, J. D., A. S. LOUGH AND R. W. LODGE, 1975. The chemical ecology of *Biomphalaria glabrata* (Say) the snail host of *Schistosomo mansomi* Sambon: the search for factors in media conditioned by snails which inhibit their growth and reproduction. *J. Appl. Ecol.,* **12**: 421–436.

WELSH, J. H., AND R. I. SMITH, 1960. *Laboratory exercises in invertebrate physiology.* Burgess Publ. Co., Minneapolis, 179 pp.

WOLBACH, R. A., H. O. HEINEMANN AND A. D. FISHMAN, 1959. Fate of intraperitoneally injected ammonia in the catfish, *Ameirus nebulosus.. Bull. Mt. Desert Island Biol. Lab.,* **4**: 56–57.

ABSTRACTS OF PAPERS PRESENTED AT THE MARINE BIOLOGICAL LABORATORY

Abstracts are arranged alphabetically by first author. Author and subject references will be found in the regular volume index, appearing in the December issue.

GENERAL SCIENTIFIC MEETINGS
August 23–27, 1976

The electric current generated by the sodium pump of squid giant axon: effects of external potassium and internal ADP. R. Abercrombie and P. De Weer.

The transmembrane current generated by the Na pump of the squid giant axon was calculated, for a variety of conditions, from the membrane conductance (measured by means of a "piggy-back" platinized-platinum electrode), and the small (1–2 mV) deplorization resulting from inhibition of the sodium pump with cardiotonic steroids. Simultaneously, the rate constant for Na efflux was determined by monitoring the efflux of previously micro-injected radioactive sodium. In a normal axon, the magnitude of the charge translocated, per ion of Na extruded, appeared to be contsant as external K was varied between 0 and 20 mM. A number of axons were pre-injected with L-arginine in order to decrease their intracellular ATP/ADP ratio *via* the arginine phosphokinase reaction. Na efflux from such axons, though digitalis-sensitive, is largely independent of the external K concentration. On the other hand, the amount of current generated per unit of Na efflux varied as a function of K_o. In K-free solutions, arginine-treated axons (i.e., with elevated intracellular ADP) showed no electrogenicity. With increasing concentrations of K_o present, however, the sodium pump of arginine-treated axons also became electrogenic, and the current generated per unit of Na efflux for these cells became comparable to that for normal axons, suggsting that electroneutral Na-Na exchange was supplanted by electrogenic Na-K exchange as the concentration of K_o was raised.

Supported by the Grass Foundation Summer Fellowship Program and by NIH grant NS-11223.

Confirmed: the existence of abundant endoplasmic filaments in Nitella. Nina S. Allen, Robert D. Allen and Thomas E. Reinhart.

Scanning electron micrographs of fixed and critical point dried internodal cells of *Nitella furcata* var. *megacarpa* have confirmed the presence of an extensive system of endoplasmic filaments 80–400 nm in diameter. These filaments correspond in size and position to the undulating endoplasmic filaments previously described as existing throughout the endoplasm by N. S. Allen (1974, *J. Cell Biol.*, **63**: 270). The force with which these undulations occur was calculated to be sufficient to drive endoplasmic streaming. These endoplasmic filaments (at or below the limits of resolution of the light microscope) as well as the thicker subcortical fibrils attached to the rows of cortical chloroplasts, can transport particles at streaming velocities (60–100 μm/cm).

Fixed, critical point dried cells were cut open and spread to show the tonoplast surface. Depending on the extent of damage from fixation and cutting, various layers of the cytoplasm could be observed. Where the endoplasm had been moved aside, the chloroplast layer could be seen, often with the subcortical fibrils intact. Where more of the endoplasm was preserved, it could be seen to consist of a network of filaments oriented predominantly in the direction of the chloroplast rows (and streaming) but containing thin transverse connections and branching points as well. The tendency of parallel endoplasmic filaments to aggregate into bundles several microns thick in places while absent in other places may have contributed to the difficulty in confirming the presence of endoplasmic filaments by transmission electron microscopy.

Supported by a NIH research grant GM 22356.

Interaction of motile blood cells of Limulus *in vitro.* PETER B. ARMSTRONG.

Contact paralysis, defined as the inhibition of pseudopodial activity over those portions of a cell's surface that lie in contact with other cells, is interesting as a mode of cell interaction important in controlling motility in cell populations. Contact paralysis has been proposed to underlie the monolayering behavior of vertebrate cells in culture: the cessation of pseudopodial activity at regions of cell contact should ensure that cells do not migrate onto the dorsal surfaces of neighboring cells. Although most of the study of contact paralysis has utilized tissue cells of vertebrates, classic studies of Leo Loeb (1920, *Washington Univ. Studies,* **8**: 3–79) on *Limulus* amebocytes in culture pioneered the concept of contact paralysis. The present study represents a reinvestigation of the contact behavior of *Limulus* amebocytes using frame-by-frame analysis of time-lapse microcinematographic records.

As described by Loeb, amebocytes do show contact paralysis of the pseudopods along those portions of their surfaces that contact other cells during the course of cellular locomotion. However, the effects of contact on cell motility is very different from that seen in vertebrate cells. *Limulus* amebocytes are only weakly prohibited from overlapping cells that they collide with. They often move bodily onto neighboring cells and move actively over their dorsal surfaces. Contact paralysis is, thus, not a sufficient condition for monolayering. Differences in the pseudopods may underlie the differences in overlapping behavior seen between vertebrate cells and *Limulus* amebocytes. The pseudopods of vertebrate tissue cells are extended in contact with the substrate, whereas amebocyte pseudopods are often protruded well above the substrate. In the latter cells, motility occurs after the extended pseudopod is lowered to contact the substrate. If, instead of glass, the pseudopod is lowered onto an adjacent cell, subsequent motility will bring one cell onto the dorsal surface of the other.

Supported in part by Cancer Research Funds of the University of California.

Pigments in the eggs of Limulus polyphemus. ERIC G. BALL.

A number of pigments are present in the ripe eggs of *Limulus*, and the following procedures have been developed for their extraction and separation. The eggs are ground and exhaustively extracted with acetone. Yellow pigments are thus removed which are mainly carotenoids as judged by their solubility properties and absorption spectrum peaks (453 and 478 nm). Extraction is next carried out with 95% ethanol until a second yellow pigment is removed. This pigment absorbs only at wave lengths below 320 nm and remains unidentified. A final extraction of the eggs is then carried out with acid 95% ethanol. These extracts are bluish-green and upon dilution with water the colored material is removed by chloroform. Silica gel is added to the chloroform solutions in amounts just sufficient to adsorb the bluish-green material, and the gel is washed repeatedly with chloroform. These washes and the original chloroform supernatant are brown in color and display an absorption spectrum with many peaks in the region 398 to 744 nm. There is some indication that these brown pigments may be derived from the blue-green material. The pigments adsorbed by the silica gel are eluted with methanol and their further purification is achieved by thin layer chromatography on silica gel plates using a chloroform-methanol mixture (9:1). A number of components are thus revealed. A major blue-green component (A) has an R_f of 0.38, absorption peaks at 378 and 670 nm, and gives a strong Gmelin test. One minor component (B) is yellow with an R_f of 1.0 and has a single absorption peak at 450 nm. It readily undergoes oxidation to a blue-green compound not unlike pigment (A). Both compounds appear to be bile pigments. Component (A) resembles biliverdin in its properties, while component (B) may be bilirubin or a closely related compound.

Supported by NIH grant AM-16793.

Unstirred layers—their effect on intestinal absorption kinetics for glycine and on sodium-glycine interactions in the marine teleost, Opsanus tau. DEREK BARKALOW AND A. FARMANFARMAIAN.

Unstirred fluid layers were disturbed in a closed loop preparation of *in vivo* toadfish midgut by use of an oscillating pump stirrer which periodically transferred incubation solution between a syringe and the gut loop. The mechanics of the stirring were arranged such that for varying stirring rates, a constant mean luminal volume of incubation solution was maintained. This prevented any appreciable changes in the surface area available for substrate transport as the frequency of stirring was altered. The aim of this stirring was to continuously provide for a homogeneous luminal solution from which substrates could be absorbed by the brush border membrane.

A kinetic analysis of the effect of stirring on net glycine absorption showed a consistent increase in glycine uptake at a stirring speed of 20 oscillations per minute (opm) *vs.* no stirring. Luminal protein concentration of terminal solutions was double for 20 opm stir *vs.* no stir, suggesting the pumping action was vigorous enough to dislodge surface proteins associated with the brush border membrane yet not so damaging as to cause a release of blood or tissue debris into the lumen. Transport constants derived from this kinetic analysis showed no significant difference in the concentration of glycine required for half maximal velocity (K_t) for 20 opm stir *vs.* no stir; however, a significant difference of approximately 80% was observed in the maximal velocity (J^{max}) for stirred *vs.* unstirred conditions.

The effect of unstirred layers on Na-glycine interactions was tested with Na^+-free, mannitol-substituted saline *vs.* a normal teleost saline containing 150 mM Na^+ at 20 opm stirring. The absence of Na^+ from the test solution produced a small but consistent reduction in net glycine absorption rate at all concentrations tested. This reduction was about 20%. No significant change was seen in K_t; but J^{max} was reduced by approximately 30%, and this was statistically significant.

Supported by funds from Rutgers University, Marine Sciences Center and Mr. N. Rutgers.

Calcium directly regulates transmitter uptake by squid brain synaptosomes. JEFFERY L. BARKER AND HARVEY B. POLLARD.

Transmitter uptake at nerve endings is believed to be an integral part of the process of synaptic transmission. Transmission is terminated, in many cases, by removal of the transmitter from the cleft by the uptake mechanism, and new transmitter stores are reassembled from the recovered species for subsequent transmission. Calcium is an important regulator in release of transmitter, and we have studied its possible role in regulation of reuptake. We report that low calcium levels enhance the high affinity uptake by 2–3 fold for a number of transmitter and transmitter precursors, including L-noradrenaline (L-NA), dopamine, serotonin, choline, GABA and glutamate. The mechanism of activation was studied in more detail for L-NA, and was found to be uncompetitive. The V_{max} was elevated from 60 to 200 pmoles/mg protein × min, while the Km was increased from 3.8 to 10 μM. The uncompetitive mechanism suggested that Ca^{++} was able to interact directly with the transmitter uptake system (permease) forming a ternary complex (Ca^{++}-transmitter-permease). This ternary complex was relatively less active than the transmitter-permease complex without calcium. In prospect, it was possible that activation in low calcium might have occurred through inhibition of the release system. However, this was inconsistent with the observed kinetics. In the latter case, noncompetitive kinetics (only V_{max} changed) would have been expected. We further tested this concept directly by assaying uptake in 100 mM K^+ (release conditions). Yet, the same uncompetitive kinetics were observed. We interpret these results to mean that lowered extracellular calcium may activate the transmitter uptake system by affecting the specific permeases directly. The physiological relevance of these observations may be found in previous reports that cells accumulate calcium during release, perhaps leading to momentary declines in Ca^{++} concentration at the cleft. Such a low calcium state would activate transmitter uptake for replenishment of transmitter stores.

Efferent mediated circadian changes in the neural activity of the Limulus *lateral eye.* R. B. BARLOW, JR., S. J. BOLANOWSKI, JR., AND M. L. BRACHMAN.

When *Limulus* is kept in constant darkness the lateral eye produces larger ERG and optic nerve responses during nighttime than during daytime. These responses to brief flashes of constant light intensity increase in amplitude at dusk, remain high during the night, decrease at dawn, and remain low during the day. The level of spontaneous activity recorded from a single optic nerve fiber is inversely related to the amplitude of the light-evoked responses. Cutting the lateral optic nerve abolishes these circadian changes in neural activity. Fibers in the proximal stump of the cut lateral optic nerve give synchronous bursts of impulses during nighttime and little or no activity during daytime. This efferent activity can be modulated by illumination of the median eyes.

The efferent input to the lateral eye substantially influences the intensity coding characteristics of the receptor units (ommatidia). During the day, the intensity-response function for a single ommatidium in the lateral eye *in situ* with optic nerve uncut is similar to the intensity-response function obtained when the optic nerve is cut. During the night, the efferent input to the eye changes the position and shape of the intensity-response function. Subtracting spontaneous activity and plotting the intensity-response functions on log-log coordinates reveals that the nighttime function is shifted vertically and to the left, suggesting an increase both in the response per absorbed photon (gain) and in the number of photons absorbed by the photoreceptors at a given light intensity. The latter effect may be caused by migration of screening pigment in or around the photoreceptors. This mechanism, however, cannot readily account for the observed changes in gain or spontaneous activity.

Supported by NIH grant EY-00667.

Comparison of facilitation and delayed release at the frog neuromuscular junction. STUART BARTON.

The magnitude and time course of delayed release and facilitation were measured at the same neuromuscular junction in solutions containing 2 to 8 mM strontium and no calcium. Raising the extracellular strontium concentration from 2 to 8 mM increased the half time of facilitation but decreased that of delayed release. When the magnesium concentration was adjusted to keep the size of the end-plate potential constant, it was sometimes found that the time course and magnitude of delayed release stayed the same but the decay of facilitation was faster in 2 than in 8 mM strontium. If delayed release and facilitation are caused only by residual calcium within the nerve terminal, then a change in the time course of facilitation should be associated with a change in the time course of delayed release. The observed difference in the behavior of facilitation and delayed release may be explained if some component of facilitations is caused by a difference between the action potentials produced by the first and second stimulus.

A difference between the behavior of the first and second action potentials has been observed in solutions containing high calcium concentrations. Soon after increasing the calcium concentration to 30 mM, the amplitude of the end-plate potential fell suddenly to about 20% of its initial amplitude, presumably because of a failure of the action potential to invade the entire nerve terminal arborization. A second stimulus given within 200 msec of the first produced an end-plate potential with an amplitude equal to that before the block occurred. The mechanism by which the second action potential overcomes the propagation block is not yet known.

This study was supported by the Grass Foundation and by the MRC (UK).

Aspects of the respiratory response of Carcinus maenas *(L.) to hypoxia and H_2S exposure.* ANTHONY J. BECKER, JR. AND BRIAN L. BAYNE.

Carcinus maenas (L.), the green crab, is an intertidal organism which frequently encounters hypoxic conditions during the tidal cycle. The present study was designed to examine the respiratory response of *Carcinus* to reduced oxygen tensions and to test the hypothesis that H_2S may affect the respiratory response. The branchial chambers of *Carcinus* were cannulated with flexible nylon tubing, connected to a pressure transducer; the animals were placed in respirometers with either sea water or a solution of 50 mg Na_2S/liter sea water. The rate and magnitude of both ventilations and reversals of flow in the branchial chambers were recorded continuously on a polygraph and the partial pressure of O_2 (PO_2) in the respirometers was measured at 15 minute intervals. Aerobic shutdown was considered to have occurred when the PO_2 levelled out. Animals were acclimated in the respirometers for 60 minutes in running sea water at 22 ± 1° C and 150 ± 10 mm Hg PO_2 prior to sealing.

Under conditions of decreasing PO_2, regardless of the presence of the sulfide ion, the rate and magnitude of both ventilations and reversals initially increase and subsequently decrease and become progressively erratic until they cease at the point of shutdown. Periodically, the water in the respirometers was replaced with water which had been partially stripped of O_2 by bubbling N_2 through a gas exchange column, thus minimizing the effects of waste product accumulation. In sea water, shutdown occurred at 23 mm Hg PO_2, while in the presence of the sulfide ion, shutdown occurred significantly (<0.005) earlier at 41 mm Hg PO_2.

These data indicate that *Carcinus* may have a mechanism for monitoring H_2S concentrations in its environment and may use the presence of the sulfide ion as a cue to impending anoxic conditions.

Ultrastructural observations on the squid giant axon and associated Schwann cell sheath. Robert A. Bloodgood and Joel L. Rosenbaum.

The ultrastructure of giant axons, isolated axoplasm and isolated axonal sheaths of the squid *Loligo pealei* has been investigated using thin sections of material fixed in 3% glutaraldehyde in 100 mM Pipes at pH 7.0 or prepared by the method of Gray (1975, *Proc. Roy. Soc. London Ser. B,* **190**: 369) by sequential exposure to 20% bovine serum albumin, 4% osmium tetroxide, 12.5% glutaraldehyde and 2% uranyl acetate.

The axoplasm contains a dense oriented array of 250 Å microtubules and 70 Å neurofilaments. Many more microtubules are seen in the material prepared by the Gray procedure than by any other fixation procedure used on the giant axon. Many projections are seen on the microtubules and often appear to interconnect adjacent microtubules. Many microtubules are seen apposed to the inner surface of the axonal membrane, even in the isolated sheath; sometimes these structures are connected by fine crossbridges. Although no microfilaments have been observed within the axoplasm or associated with the axonal membrane, numerous 40–50 Å microfilaments occur within the Schwann cells wrapping the giant axon. These microfilaments are identical in appearance to the actin-containing microfilaments described in other nonmuscle cells, occur in bundles, and in association with the Schwann cell membranes. The Schwann cell layer is very fragile and release of filaments from disrupted cells may account, at least in part, for the observation by other workers of actin and microfilaments within glycerinated axons and isolated axoplasm of the squid.

The firm, gel-like consistency of isolated axoplasm is stable for many hours in 100 mM Pipes, pH 7.0 at 4° C. The axoplasm solubilizes within 10 minutes in the presence of 5 mM calcium or 0.5 M KCl, both treatments resulting in a loss of neurofilaments. The axoplasmic structure is stable in 5 mM calcium in the presence of 1 mM N-ethyl maleimide at 4° C for 24 hr although electron microscopy shows the loss of all microtubules. These observations suggest that the axoplasmic network is held together by neurofilaments and not microtubules and that calcium induced solubilization occurs through a mechanism sensitive to sulfhydral poisons. A calcium-activated, p-hydroxymercuribenzoate-sensitive protease has been reported from squid axoplasm (Orrego, 1971, *J. Neurochem.,* **18**: 2249).

R. A. Bloodgood is the recipient of a Steps Toward Independence fellowship from the Marine Biological Laboratory.

In vitro *studies on the physiology of mitosis in oocytes of* Chaetopterus. Gary G. Borisy and Shinya Inoué.

At 21° C, oocytes of *Chaetopterus pergamentaceous* enter anaphase ten minutes after fertilization. In the first half minute of anaphase, chromosomes are stretched poleward to twice their metaphase lengths; they then snap apart and move poleward at *ca.* 4 μm/min. The spindle length decreases from 35 to 32 μm as the chromosomes are stretched, remains constant for most of anaphase, then decreases progressively to *ca.* 20 μm during late anaphase. Spindles displaying some of these *in vivo* properties were prepared *in vitro* by mechanically lysing oocytes in isolation media containing 0.1 M Pipes pH 6.9, 2 mM GTP, and 5–100 mM EGTA. In metaphase cells, the hypotonic isolation media induced chromosomal spindle fiber shortening as evidenced by chromosome stretching.. After spindle isolation, chromosomes remained stretched as long as birefringence persisted. As chromosomal fiber birefringence diminished, with time or after cooling, the chromosomes retracted to their metaphase lengths. Surprisingly, sister chromatids in spindles isolated in mid- to late-anaphase also moved back together as spindle fiber birefringence decayed. Retrograde motions in metaphase and anaphase isolates occurred either as spindle length decreased or at constant spindle length as chromosomal fibers depolymerized. The forces maintaining chromosome stretching and separation are therefore microtubule-dependent. In metaphase and anaphase isolates, chromosomes retracted individually at varying rates. Therefore, forces were exerted on individual chromosomal fibers. Exogenous porcine brain tubulin (5 mg/ml) sustained birefringence and chromosome stretching and appeared to block chromosome movement, forward or backward. *In vitro* anaphase was attempted using endogenous tubulin by lysing populations of oocytes at high cell densities. Some poleward movement (1–2 μm in 2–3 min) of mid-anaphase chromosomes was observed in the isolates at constant spindle lengths but the movement did not continue.

Grant support from NIH GM-21963 (G.G.B.) and GM-23475 and NSF BMS-00473 (S.I.)

Histone messenger RNA expression in echinoid hybrid embryos. Barbara Braun, Barbara W. Nagle and Laurence Kedes.

Templates for histones are the major mRNA's synthesized during the cleavage stage of sea urchin embryogenesis. Radiolabelled histone mRNA's from different species exhibit distinctive patterns of electrophoretic mobility. We have compared histone mRNA's synthesized by interspecies echinoid hybrids with parental mRNA patterns in order to analyze the contributions of the maternal and paternal genomes to histone mRNA synthesis during cleavage. Three species of Echinoderm were used: *Arbacia punctulata* (A), *Echinarachnius parma* (E), and *Lytechinus pictus* (L). Two hybrids were obtained in sufficient quantity: E eggs and A sperm (EA), and A eggs and L sperm (AL). These crosses required three critical conditions: first pretreatment of eggs with dithiothreitol (10 mM, pH 9.1) to remove the vitelline membrane; secondly, utilization of a high sperm concentration; and thirdly, elevated pH (8.5–9.0). None of these conditions alone or in combinations of two activated the eggs. RNA of the hybrid and control embryos was labeled *in vivo* with ^{3}H-uridine. The isolated polysomal RNA was analyzed by polyacrylamide gel electrophoresis and labelled RNA's were detected by fluorography. EA hybrids labelled from 2–6 hours and from 2–12 hours post-fertilization exhibited a distinct pattern of bands similar to that of the maternal parent and lacked bands characteristic of the paternal species. The mobilities of the bands suggests that E embryos actively synthesize histone templates. The mRNA's of the six hour AL embryos were a composite of the parental strains. At least two RNA's had mobilities of maternal templates and two had mobilities of paternal templates. Several maternal and paternal RNA's were not labelled in the hybrid embryos. Several RNA bands had identical mobilities in the parental RNA's and thus, their origin in the hybrids could not be assigned. This result suggests that expression of several paternal genes in the AL hybrid occurs within the first six hours of development.

This research was supported by NSF Grant #PCM76-09315; BWN is an NIH predoctoral trainee; and BB is supported by an NIH research grant.

A pharmacological and electrophysiological study of ciliary innervation of the gill of Mytilus edulis *(Bivalvia).* Edward J. Catapane.

Previous work from our laboratory has shown that lateral ciliary activity can be controlled from the visceral ganglion. Applying serotonin (10^{-6}–10^{-3} M) to it results in cilio-excitation, while applying dopamine (10^{-6}–10^{-3} M) results in cilio-inhibition. Recording electrically from the branchial nerve, which innervates the gill, while simultaneously measuring ciliary beating rates strobomicroscopically, demonstrated that cilio-inhibition was associated with an increase in frequency of action potentials (15–50 μV) arising from the visceral ganglion, from a basal rate of less than 10 spikes/min to generally more that 30/min. In addition to dopamine, this response could be initiated by applying epinine, epinephrine and amphetamine to the visceral ganglion in concentrations ranging from 10^{-6}–10^{-3} M. Pretreating the ganglion with ergonovine blocked these responses. Superfusion of the ganglion with serotonin (10^{-6}–10^{-3} M) which increases ciliary beating rates was previously shown to depress basal spiking rates. In addition to this it has now been demonstrated that high ciliary beating rates, whether induced by serotonin applications to the ganglion or occurring spontaneously, are also associated with smaller amplitude bursting (less than 5 μV) recorded from the branchial nerve. The responses caused by serotonin can be blocked to varying degrees by preteating the ganglion with bromolysergic acid diethylamide, methysergide, bufotenine or ergonovine in concentrations ranging from 10^{-6}–10^{-4} M. This study supports previous suggestions of our laboratory regarding a reciprocal serotonergic-excitatory and dopaminergic inhibitory innervation of gill cilia originating from the visceral ganglion and possibly from the cerebral ganglion. It has also demonstrated the existence of small cilio-excitatory potentials.

This work was performed under a Grass Foundation Fellowship in Neurobiology.

Allozyme variation between two marshes and possible heterozygote superiority within a marsh in the bivalve Modiolus demissus. RICHARD E. CHAISSON, LESLIE A. SERUNIAN AND THOMAS J. M. SCHOPF.

The ribbed mussel *Modiolus demissus* is a common macro-inhabitant of eastern North American estuaries. Specimens of *Modiolus* of two size classes (mean-4.5 and 8.0 cm) were collected from near the mouth, the middle, and the head of Wild Harbor salt marsh, Cape Cod, Massachusetts (1 km long, 0.2 km wide). Allele frequencies at the tetrazolium oxidase (To) locus were scored in 61–95 animals in each size class from each locality using polyacrylamide gel electrophoresis and histochemical staining. The data were compared with similarly collected data from Little Sippewissett salt marsh, four miles to the south.

Within each marsh there are no significant size- or locality-dependent differences in allele frequencies at the To locus. However, there is a significant difference in values of p (frequency of slow migrating allele) and q (frequency of fast migrating allele) between the two marshes. At Wild Harbor p is 0.23 and q is 0.77, whereas at Little Sippewissett p is 0.30 and q is 0.70. Therefore, animals at spatially separate but similar habitats have different allele frequencies for this polymorphic locus.

At Wild Harbor there is departure from Hardy-Weinberg expectations for heterozygous animals. In the lower marsh D (heterozygotes observed—heterozygotes expected / heterozygotes expected) is $+0.018$ for small animals and $+0.185$ for large animals. In the upper marsh D is -0.142 for small animals and $+0.165$ for large animals. Thus, as animals in these localities increase in size, the proportion of heterozygotes increases in the population. This indicates the possibility of heterozygote superiority at the To locus.

Completed in the Experimental Invertebrate Zoology course with partial support of NSF grant GB-30870 (to Schopf). We thank Dr. J. P. Grassle and K. W. Kaufmann for advice.

Pulsed nuclear magnetic resonance studies of squid giant axon. DONALD C. CHANG.

We have measured the spin-lattice relaxation time (T_1) and spin-spin relaxation time (T_2) of water protons in squid giant axons and extruded axoplasm with a pulsed NMR technique called spin-echo. All measurements were done with a spin-lock CPS2 Spectrometer. There are two unique features of this study: first this is the first NMR study of a single fully differentiated cell; and secondly, this study offers a direct test for an interpretation which proposes that the shortening of relaxation times in biological systems is caused by the magnetic field inhomogeneity arising from the susceptibility difference between the membranes and the cytoplasmic water. The relaxation effect of membrane on the cytoplasmic water can be evaluated by comparing the relaxation times of the axon with those of the axoplasm. The results of measurements showed the following. First the T_1 of water proton in axon is reduced by a factor of two, in comparison to that of sea water. The reduction in T_2 is about a factor of eight. These reductions are significant. Secondly, the relaxation times are almost the same between the axon and the axoplasm. This indicates that the susceptibility difference between the membrane and water has a negligible effect in shortening the T_2 of water protons. Thirdly, a dead axon kept in sea water at 2° C for over one day has a longer T_2, indicating that the axoplasm in the living axon is more "structured." Fourthly, the extruded axoplasm stored in a glass tube for several days at 2° C gives similar relaxation times as fresh axoplasm. This suggests that the change of the structure of axoplasm must depend on the electrolyte concentration.

Are intracellular calcium ion injections equivalent to light adaptation in the ventral photoreceptors of Limulus? J. S. CHARLTON AND A. FEIN.

Single ventral photoreceptors of *Limulus* were impaled by two electrodes, one containing calcium ions. Photoreceptors were iontophoretically injected with Ca^{++} by passing current between the two intracellular electrodes. Cells were desensitized by the intracellular injection of calcium ions or by light adaptation. The responses (late receptor potential) to brief flashes of light were obtained during interruptions of the injecting current or the adapting light. Responses (controls) were also obtained from the dark-adapted receptor both before desensitization and after the receptor had recovered from the Ca^{++} injection or the light adaptation. It was found that for equal threshold elevations produced by light adaptation or Ca^{++} injection, the photoresponses to the same stimulii had similar response waveforms. The latencies of the responses obtained during both light adaptation and Ca^{++} desensitization were found to be shorter than the latencies of the controls. Desensitization by either method yields responses of less variability than controls. For an equal desensitization, the photoreceptor has a similar light intensity-response characteristic whether desensitized by calcium ions or light adaptation. Under the conditions of these experiments, it appears that the intracellular injection of calcium ions is roughly equivalent to light adaptation. However, on the average, the responses obtained during light adaptation are of shorter latency than those obtained during calcium desensitization.

Separation of blastomeres of Spisula solidissima *embryos and comparison of RNA and protein of the isolated blastomeres.* CLARISSA M. CHENEY, MADELYN M. BARAN AND JOAN V. RUDERMAN.

Using two techniques for separating cell types of *Spisula solidissima* embryos, we have separated embryos at the two-cell stage and compared RNA and protein of the isolated cell types. To separate the cells, the embryos are demembranated immediately after first cleavage by two washes in 0.52 M NaCl–0.02 M Tris (pH 8) and then suspended in 0.85 M sucrose–0.02 M Tris (pH 8). EDTA (pH 8) is added to a final concentration of 0.0013 M. The cells dissociate and can be isolated by the following techniques.

To isolate AB blastomeres, the dissociated cells are poured gently through a 26 μ pore size Nitex mesh. The AB blastomeres pass through the mesh. The CD blastomeres, together with any unfertilized eggs or undissociated embryos, are retained by the mesh. To isolate both AB and CD blastomeres, a sucrose density step gradient made of these six sucrose solutions is used: 0.75 M, 0.713 M, 0.678 M, 0.638 M, 0.60 M, and 0.563 M. All sucrose solutions contain 0.13 M NaCl and 0.02 M Tris (pH 8). The dissociated cells are added to an equal volume of 0.52 M NaCl–0.02 M Tris (pH 8) and layered on top of this gradient. The gradients are spun at low speed on a clinical centrifuge for three minutes. Two sequential gradients give greater than 90% purity of the cell populations.

Total cell RNA was extracted from the isolated blastomeres and translated in a cell-free wheat germ system. The ^{35}S-methionine labelled protein product was analyzed by SDS-acrylamide slab gel electrophoresis. Autoradiography of this one-dimensional gel showed no obvious differences between the blastomeres. Electrophoresis using an O'Farrell two-dimensional gel showed some quantitative and possible qualitative differences between these translation products. Isolated blastomeres were also cultured for one and one-half hours in the presence of ^{35}S-methionine and examined by one-dimensional electrophoresis. This gel showed differences between the two-cell embryos and the isolated blastomeres but did not show differences between the AB and CD blastomeres.

This work was supported by NSF grant #PCM 76–09315. J.V.R. is a Jane Coffin Childs post-doctoral fellow. C.M.C. and M.M.B. are N.I.H. predoctoral trainees.

Phycobiliprotein synthesis in protoplasts of the unicellular cyanophyte, Anacystis nidulans. JILL C. COSNER AND ROBERT F. TROXLER.

Phycobiliproteins are accessory pigments found in the photosynthetic apparatus of the Cyanophyta, Rhodophyta and Cryptophyta. Allophycocyanin and phycocyanin are the principal phycobiliproteins in *A. nidulans*. These pigments are comprised of subunit polypeptides to which the bile pigment chromophore, phycocyanobilin, is covalently attached. The present work describes phycobiliprotein synthesis in protoplasts obtained from this organism.

Stable and metabolically active protoplasts were prepared by incubation of algal cells at 37° C with 0.1% lysozyme in 0.5 M mannitol and 0.03 M sodium phosphate buffer, pH 6.8. Maximum protoplast yield was obtained after incubation for 1–2 hours. Subsequent to enzymatic digestion of cell wall material, protoplasts were incubated at 37° C with L-[U-^{14}C] leucine (300 μCi/μmole; 5 μCi/30 ml protoplasts) in 0.5 M mannitol and the growth medium of Kratz and Myers, Medium C. At intervals, cold trichloroacetic acid (TCA) insoluble material from protoplast lysate was prepared and assayed for radioactivity. Incorporation of radiolabeled leucine was linear for two hours. Preparation of hot TCA insoluble material demonstrated leucine incorporation into the polypeptide backbone of protein from *A. nidulans* protoplasts.

Protoplasts were incubated as above for 30 minutes, divided into three equal portions to which was added: distilled water (control), unlabeled leucine (final concentration 1 mM), and chloramphenicol (final conc 150 μg/ml). Control protoplasts continued to incorporate leucine into protein for an additional 1.5 hours. Incorporation of radiolabeled leucine ceased in protoplasts incubated with unlabeled leucine. Interestingly, the radioactivity in protoplast protein following incubation with chloramphenicol decreased significantly, indicating that protein turnover had occurred.

Protoplasts were incubated with radiolabeled leucine for three hours. Phycobiliproteins in the lysate were purified on a brushite column developed with phosphate buffers (pH 6.8) of increasing ionic strength. The specific radioactivity of eluted phycocyanin ($E_{620/280} > 4$) was 47,900 cpm/mg and that of allophycocyanin ($E_{650/280} \leqq 2$) was 93,500 cpm/mg. These data demonstrate phycobiliprotein synthesis in *A. nidulons* protoplasts *in vitro* and provide a model system for studying regulation of protein synthesis in blue-green algae.

Supported by National Science Foundation grant no. BMS 74–20633.

"Fertilization product" protease of Arbacia punctulata: *Ca^{++} effects, protein and synthetic substrates.* JOHN G. CSERNANSKY, EMILY S. HARRIS, REID LIFSET, ALBERT GROSSMAN, WALTER TROLL AND MILTON LEVY.

The *Arbacia* protease, secreted at fertilization in a particulate form, was solubilized and assayed to determine its requirement of Ca^{++} for activity. Succinylated protamine was digested by the sea urchin protease at 37° C and pH 8.0. The appearance of primary amino groups measured fluorometrically was taken as a reflection of proteolysis. Alternatively, Bz-Phe-Val-Arg-p-nitroanilide was digested by the sea urchin protease at 23° C and pH 8.2. Cleavage of the arginyl-*p*-nitroanilide amide bond released *p*-nitroaniline, a chromophore. Increasing absorbance at 405 nm over time reflected protease activity.

Treatment of the particulate form of the enzyme with 10 mM EGTA (pH 8.0) produced a visible precipitation and apparent loss of activity, partially reversible when excess Ca^{++} was added. Electron microscopy clearly showed flocculent aggregation of the protease particles. After solubilization in a KCl-CaCl$_2$-butanol-glycerol-borate buffer (pH 8.0), treated with 10 to 60 mM EGTA failed to cause loss of protease activity. The solubilized, as well as the particulate protease, was inhibited with soybean trypsin inhibitor (10 μg/ml). Loss of activity in the particulate was attributed to physical obstruction of the active site caused by particle aggregation. The activity of the protease, once solubilized, is independent of Ca^{++} concentration. The data suggest that Ca^{++} participates in stabilization of a particulate superstructure, thus maintaining a protective control on an enzyme which has a broad spectrum of substrate specificity.

We acknowledge the New York University School of Medicine Honors Program for support.

Structure of nerve terminals in frog muscle after prolonged treatment with brown widow spider venom. D. A. DAMASSA, T. L. DAVIS, D. M. SHOTTON, J. E. HEUSER, D. PUMPLIN AND T. S. REESE.

Black or brown widow spider venoms are thought to induce discharge of synaptic vesicles from frog motor nerve terminals and to block the subsequent reformation of synaptic vesicles.

If synaptic vesicle membrane is added to the plasmalemma during discharge, and not recovered, there should be an increase in the surface area of nerve terminals at later stages of the venom effect. We looked for such an increase by measuring, in electron micrographs, the perimeters of cross sections through nerve terminals in frog cutaneous pectoris muscles. Nerve terminals on muscles treated for two hours with brown widow spider venom in Ca^{++}-free Ringer's which contained 5 mM Mg^{++} were compared with control muscles incubated for a similar period in the absence of venom prior to fixation in aldehydes. No difference in perimeter was detected between 53 control (6.4 ± 0.3 μm s.e.) and 46 venom-treated (6.4 ± 0.3 μm) terminals from several muscles. As expected, stimulated terminals contained almost no synaptic vesicles (3 ± 0.7 per cross section), compared to controls (101 ± 10), but we were surprised to find that they contained many large membrane sacs resembling the cisternae seen in electrically stimulated nerve terminals. Estimation of the amount of cisternal membrane indicated that it could account for the lost synaptic vesicle membrane. Tissue from the same experiments was also freeze-fractured, which showed that many of the intraterminal cisternae have very few particles in their membranes. Patches devoid of particles were also found in the surface membrane of venom-treated terminals. When earlier stages of venom action were examined with the freeze-fracture technique, a few images were obtained of deep invaginations of the surface membrane terminating in particle-free sacs. These findings suggest that some cisternae might arise directly from the surface membrane.

Blood pressure and locomotion in bluefish (Pomatomus saltatrix). A. B. DuBois, R. S. Fox, A. N. Wilner and R. H. Lambertsen.

Previous experiments showed that the pressure distribution along the body surface of bluefish swimming at 4 miles per hour is similar in magnitude to the transmural hydrostatic pressure gradient in a land animal. From this, it was postulated that the bluefish circulation might be able to withstand gravity even though it had never been exposed to its full force. Four bluefish were placed on a V-board in air with water perfusing the gills. Blood pressure was measured in the ventral aorta. The V-board was tilted at 30° and 60° head up for 35 minutes. The blood pressure, though slightly reduced, remained stable. All four fish survived the tilting and swam normally afterward. It was concluded that the circulation of bluefish withstands gravity as well as that of land animals. We extended the data on the relationship between body surface pressure and speed by two methods. Wooden spindles were made with contours similar to those of the back and side of bluefish. These were placed in a long wooden tunnel described in previous work and subjected to water flow at various speeds. The surface pressures were similar to those on the swimming bluefish. In addition, live bluefish were placed in the same tunnel with small, flat pressure gauges mounted on the tail surfaces. The thrust of the tail was proportional to pressure × tail area × sine of the tail angle. This thrust was approximately equal to the thrust calculated from an accelerometer on the fish, as thrust equals mass × acceleration. In conclusion, the forces of thrust, acceleration, and drag appear to have been sufficient to prepare the body of bluefish for tolerance to gravity.

A general method for the study of fusion of lipid vesicles using a calcium-sensitive dye, arsenazo III. Philip Dunham, Paula Babiarz, Alan Israel, Aurelio Zerial and Gerald Weissmann.

Membrane fusion is central to cellular processes such as endocytosis, secretion, fission, and formation of syncytia. A rigorous definition of fusion requires that two aqueous, membrane-bounded compartments become confluent with each other without access of the contained solutes to a third, external compartment. We have devised a method for measuring fusion according to this criterion using the metallochromic calcium-sensitive dye, arsenazo III (AIII), entrapped in multilamellar liposomes (molar per cent: phosphatidyl choline, 90; dicetylphosphate, 10). Two preparations of liposomes were used: one containing AIII and Ca, another containing the Ca chelator EGTA. In mixtures of these liposomes, interaction of EGTA with AIII Ca (AIII·Ca to AIII) is measured by a shift in the absorption spectrum of AIII from blue to red (decreased absorbance at 660 nm). *Fusion* of liposomes (but also lysis and diffusion of solutes across the membranes) would result in the absorbance decrease. The contribution of *lysis* was determined by rechromatography (Sepharose 2B): solute released by lysis is retained by the column (solute in liposomes emerges in the void volume). The contribution of *diffusion* was determined from the absorbance decreases in suspensions of AIII·Ca liposomes alone in medium containing free EGTA. Fusion mixtures were prepared containing the "fusogens" lysolecithin or retinol. After incubation (5–20 hours, 37° C) and rechromatography of the liposomes, measurements were made of total dye, fraction of dye converted from AIII·Ca to AIII, and total lipid. After correction for lysis and diffusion, values were obtained for per cent fusion (volume of AIII liposomes confluent with EGTA liposomes). In five experiments per cent fusion promoted by lysolecithin (200 μg/ml) was 23.5. Retinol (300 μg/ml) caused 15.0 per cent fusion. With the membrane stabilizing agent hydrocortisone incorporated into the liposomal membranes (1.0 molar per cent) fusion induced by both fusogens was reduced 2-fold or more. Several agents were tested as endogenous fusogens incorporated in the membranes (molar per cent: lysolcithin, 1–8; diethylstilbestrol, 1–3; phosphatidylserine, 1–10). No fusion was observed in five hours at 37° C.

Observations on the effects of light and temperature on the apparent neurosecretory cells of Mytilus edulis. DAVID W. ELVIN.

The behavior of neurosecretory cells in the cerebral and visceral ganglia of sexually mature *Mytilus edulis* was studied with respect to several light and thermal regimes. Mussels collected during June and July were kept two weeks under combinations of warm (22° C) or cold (4° C) temperatures with either continuous light or darkness. The animals were then subjected to the standard paraldehyde fuchsin technique for neurosecretory material. More material accumulated in the 7 μ cells of the ganglia of cold acclimated animals, and this accumulation probably results from a reduced release of neurosecretory material.

When animals kept in the dark at 4° C were given a 16° C temperature increase for 5 hours each day, the cerebral ganglia showed no decrease in stored neurosecretory material, but the amounts in the scattered cells of the visceral ganglia were lower. It is suggested that the visceral ganglion may be more sensitive to temperature changes than the cerebral. Animals acclimated under a 4° C regime with a 12 hour photoperiod were also subjected to a similar thermal shock. This time both the visceral and cerebral ganglia demonstrated less neurosecretory material, implying that a thermally initiated neurosecretory release by the cerebral ganglia might be enhanced by small amounts of light. A photometer designed to measure the light penetrating the shell and mantle of a closed mussel indicated this value to be 10% of the amount impinging on the surface.

Ganglia were removed and the polypeptides separated using SDS-polyacrylamide gel electrophoresis. A diffuse band of 8900 daltons was found to be stronger in those ganglia from 22° C regimes than in those from 4° C. This band is not associated with the prominent 7 μ neurosecretory cells and must be related to the contents of other cells. However, a strong polypeptide band of 12,000 daltons is associated with the concentration of 7 μ neurosecretory cells of the anterior portion of the cerebral ganglion.

This study was partially supported by an Institutional Grant from the University of Vermont.

Identification of Na conduction noise in squid axon. H. M. FISHMAN, L. E. MOORE AND D. J. M. POUSSART.

Under normal ionic conditions in squid axon, Na conduction fluctuations are masked by significantly larger fluctuations in the K conduction system. Various means of measuring Na conduction noise were explored. The most effective ionic condition for observation of Na noise is an internal perfusion with a solution of 0.29 M CsF, 0.4 M sucrose, 5 mM Tris-Cl and sea water externally. Step clamps from a hyperpolarized holding potential show virtually unobscured inward Na currents. Spectral analysis of spontaneous current fluctuations from small, isolated, voltage-clamped patches at 5° C, under these conditions, yield a residual spectrum at hyperpolarized holding potential. Upon depolarization, an additional noise component is measured in the 100–1000 Hz range. The intensity of this component reaches a maximum and then decreases upon further depolarization. The potential of maximum noise intensity correlates with that of maximum inward current in step clamp. The addition of tetrodotoxin (TTX, 1 μM) to the external sea water of a patch eliminates this additional component. Preliminary experiments in axons treated internally with pronase to alter Na "inactivation" do not show the above features. The following observations strongly suggest that a significant component of the total current fluctuation reflects Na conduction: 1) the condition of measurement—in which the K system current and noise are effectively suppressed and the Na system is preserved; 2) the spectral intensity behavior with potential—which parallels step clamp measures of Na current; 3) the elimination of property (2) after treatment with pronase—which indicates a relationship, in part, to Na "inactivation"; and 4) the disappearance of the noise component upon application of TTX—which indicates that this component is not induced noise in K channels. Others claimed erroneously to have measured Na noise in the preesnce of high internal [K] and with tetraethylammonium (TEA) as a K "blocking" agent. However, we have reported previously that TEA, in the presence of high $[K]_i$, induces substantial K channel noise which obscures Na noise and makes its identification unlikely.

Aided by NIH grants NS-11764, NS-80409 and CNRC grant A5274.

Purification and characterization of smooth muscle actin-binding protein. Walter E. Fowler and Thomas D. Pollard.

We isolated an actin-binding protein of approximately 250,000 daltons subunit molecular weight from chicken gizzard. The protein is extracted in a low ionic strength buffer containing Triton X-100 and purified by fractional ammonium sulfate precipitation followed by gel filtration on 8% agarose in a KCl/KI buffer. This yields per gram of gizzard about 1 mg of protein consisting of a single major polypeptide by gel electrophoretic analysis. The gizzard actin-binding protein elutes from the gel filtration column in two peaks: an aggregated fraction in the void volume and a "monomeric" fraction having a Stokes radius of about 7 nm. Electron microscopic examination of the negatively stained "monomer" fraction reveals spherical particles about 8–9 nm in diameter and no filamentous material. Providing that the hydrated protein is also spherical, the Stokes radius of the "monomer" form indicates that it consists of more than one 250,000 dalton subunit. A 3:1 mixture of rabbit muscle actin and gizzard actin-binding protein in a sucrose solution containing MgATP forms a gel. Controls containing either protein alone do not gel. By pelleting the gelled mixture in the ultracentrifuge, we showed that the actin-binding protein does, in fact, bind to actin. We used a gel filtration assay to study the binding of the actin-binding protein to liposomes. The results were consistent with the existence of actin-binding protein-liposome interaction, but cannot be considered to be conclusive. This gizzard actin-binding protein is probably identical to the protein, previously called filamin, isolated by Wang, Ash and Singer (1975, *Proc. Nat. Acad. Sci.*, 72: 4483–4486).

Histone gene organization of six marine invertebrates. Nevis Fregien, Mark Marchionni and Laurence Kedes.

We have examined histone gene organization in chromosomal DNA of six marine invertebrates. DNA was cleaved with restriction endonucleases, electrophoresed on agarose gels and blotted onto nitrocellulose filters. The filter-bound DNA was hybridized *in situ* with labelled sea urchin (*Strongylocentrotus purpuratus*) histone genes obtained from *E. coli* chimeric plasmids pSp2 and pSp17. Fragment lengths of hybrids were determined by autoradiography. In the case of a tandemly arranged repetitive gene, the sums of fragment lengths generated by every endonuclease are identical and equal to the unit repeat length.

Unit lengths in kilobase pairs (1 kb = 1000 base pairs) determined by digestion of chromosomal DNA with Hind III or Hind III plus Eco RI are: 7.0 kb for *Arbacia punctulata;* 4.1 kb for *Limulus polyphemus;* 4.5 kb for *Spisula solidissima;* 5.2 kb for *Chaetopterus pergamentaceous;* 6.1 kb for *Echinarachnius parma;* and 6.3 kb for *Crassostrea virginica.*

Arbacia punctulata DNA has one Hind III site and one Eco RI site per 7.0 kb gene repeat. Eco RI plus Hind III generated 4.6 kb and 2.4 kb fragments. DNA representing genes for each of the five histone proteins of *S. purpuratus* were prepared from Hha I fragments of pSp2 and pSp17. The *A. punctulata* repeat unit hybridized with each of the five gene probes. The H1 gene is assigned to the 2.4 kb segment and the H2A, H2B, H3 and H4 genes to the 4.6 kb segment.

E. parma histone gene repeat unit has two Hind III sites which generate fragments of 3.8 kb and 2.3 kb. The 3.8 kb fragment has the single Eco RI site located 0.6 kb from an end. The H3 gene is assigned to the 2.3 kb fragment and the H1, H2A, H2B and H4 genes to the 3.8 kb fragment. We conclude that some major features of histone gene organization, including tandem repeat units each coding for all five hitsone proteins, are common to all six genomes examined.

This work supported in part by NSF grant #PCM76–09315 and the Veterans Administration.

Repetitive sequence representation in sea urchin embryo nuclear RNA. CYNTHIA FRENCH AND TOM HUMPHREYS.

The middle repetitive sequence representation of heterogeneous nuclear RNA was measured in sea urchin embryos (*Arbacia punctulata*) by RNA-driven hybridizations of purified nuclear RNA with radioactively labeled, middle repetitive tracer DNA. The middle repetitive DNA tracer was prepared by renaturation of total DNA to Cot 20, isolation of duplexes on hydroxyapatite and nick translation with DNA polymerase I. The reactivity of the middle repetitive tracer after hybridization with excess whole cell DNA was 55%. The major hybridization occurred as a single pseudo first order transition at an RNA Cot between 5 and 100 to a plateau of 10.5% of the tracer DNA with blastula RNA and 6% with pluteus RNA. Correcting for the two strands and reactivity of the tracer, the data suggests that 38% of the middle repetitive sequences of the DNA were represented in the blastula RNA while 22% were in pluteus RNA. These results show that a significant portion of the middle repetitive sequences are transcribed. Indeed it appears that the per cent expression of the repetitive sequences in the genome are quantitatively similar to the per cent of the single copy sequences which are expressed. This result is possible only if all copies of most of the repetitive sequences are excluded from transcribed regions while many of the copies of each transcribed repetitive sequence must be active in order to produce known interspersion of repetitive and single copy sequences in the nuclear RNA. These results demonstrate that tracer experiments with middle repetitive DNA are as feasible as previous studies with single copy tracer and will contribute to an understanding of the role of repetitive sequences in cell function and development.

This work was supported by NSF grant #PCM76–09315.

Investigation of a poly(A) minus nonhistone messenger RNA in sea urchin eggs and embryos. ERIC A. FYRBERG AND JOAN V. RUDERMAN.

The localization and developmental fate of a particular nonadenylated (poly(A)minus) egg mRNA encoding a 70–80,000 mol wt protein has been investigated using an *in vitro* translation assay. RNA was isolated by the SDS-phenol-chloroform procedure and translated in a wheat germ cell-free protein synthesizing system containing ^{35}S-methionine. The *in vitro* translation products were analyzed by SDS-polyacrylamide slab gel electrophoresis and autoradiography. RNA isolated from the post-mitochondrial supernatant of the unfertilized egg encodes a wide variety of proteins and in particular directs a substantial amount of incorporation into a 70–80,000 mol wt. protein. Parallel analyses of the translation products encoded by RNA extracted from eggs and developing embryos demonstrate that this mRNA is present in two cell and sixteen cell embryos but is not detectable in the translation product encoded by sixty-four cell embryonic cytoplasmic RNA. These results suggest that this mRNA is selectively degraded between the sixteen and sixty-four cell stage. Examination of the translation products encoded by egg and sixteen cell poly(A) plus and poly(A) minus RNA (separated by oligo (dT) cellulose column chromatography) shows that virtually all of this particular mRNA is poly(A) minus, in both the egg and embryo and suggests that it is not adenylated following fertilization.

We have attempted to determine whether this mRNA is used to direct protein synthesis in the egg or if it is a stored, maternal mRNA. Polysomal, monosomal, and subribosomal fractions were obtained by sucrose gradient sedimentation of egg homogenates and the extracted RNA was translated. Polysomal and monosomal fractions are enriched for this mRNA relative to histone mRNA. These results suggest that this mRNA is translated in the unfertilized egg. Direct confirmation by analysis of proteins synthesized by the egg *in vivo* is under way. *In vivo* labelled embryonic proteins include a species which co-migrates with the 70–80,000 mol wt. egg RNA encoded protein, suggesting that the protein may be synthesized by embryos.

This work was supported by grant #PCM76–09315 from the National Science Foundation. J.V.R. is a Jane Coffin Childs post-doctoral fellow.

Receptor types and photopigments in the eel retina: an ERG analysis. James Gordon, Robert Shapley and Ehud Kaplan.

The electroretinogram (ERG) of the eel *Anguilla rostrata* was measured from excised eyecups of dark-adapted animals. Spectral sensitivity was determined from intensity-response curves at fourteen wavelengths of monochromatic light over the range 450–650 nm. ERG waveforms at constant response amplitude were similar in time course, and intensity-response curves were parallel for all wavelengths for stimulus intensities below 0.1 μW/cm^2. These results imply that a unitary spectral mechanism is involved in this range, presumably the rod photoreceptors. The wavelength of maximum sensitivity of the rods was 520 nm. The spectral sensitivity of the ERG in this range approximately fit the optical density measurements made by Carlisle and Denton (1959, *J. Mar. Biol. Ass. U. K.*, **38**: 97), and Beatty (1975, *Vision Res.*, **15**: 771), for the visual pigment of rods in the yellow eel. This pigment is a porphyropsin peaking around 520 nm. Light and electron microscopy revealed a class of cone photoreceptors in the eel which were about 1% as numerous as the rods. The cone spectral sensitivity in the ERG of the eyecup preparation was measured with flicker photometry against a 520 nm background. The cone function peaked at longer wavelengths but still probably had some rod contamination. In the ERG of the isolated retina, after all the rod pigment had been bleached, there were two peaks in the spectral sensitivity function around 450 nm and 560 nm. This implies the existence of at least two cone mechanisms, a short wavelength cone and a long wavelength cone, in the yellow eel.

This work was supported by the National Eye Institute through grants EY-1472 and EY-188. E. Kaplan was supported by a NIH Postdoctoral Fellowship.

Simultaneous recording from twelve neurons in the supraesophageal ganglion of Balanus nubilus *using a new potential sensitive dye.* A. Grinvald, B. M. Salzberg, L. B. Cohen, K. Kamino, A. S. Waggoner, C. H. Wang and D. Ti.

Using giant axons from the squid, *Loligo pealii,* we have tried 113 additional dyes in a search for larger changes in absorption or fluorescence that depend upon potential. One of these, 5-[(1-γ-Triethylammonium sulfopropyl-4(1H)-quinolylidene)-2-butenylidene]-3-ethyl-1, 3-oxazolone (NK2367), a merocyanine-oxazolone, had a signal-to-noise ratio twice as large as the best obtained previously. We used this dye in an attempt to monitor activity in neurons of the barnacle ganglion, collecting light from the individual neurons at the objective image plane using light-pipe photodiode combinations, where the diameter of the light pipes was slightly larger than the images of the cell bodies. We were able to monitor action potentials in twelve neurons simultaneously. Two were spontaneously active, and eight made antidromic action potentials when a root was stimulated with a suction electrode. Under the best circumstances we could also monitor large postsynaptic potentials. Photodynamic damage was undetectable in these experiments. We hope that this apparatus can be expanded so that most of the neurons in the ganglion can be monitored simultaneously.

Supported in part by Public Health Service grants NS–08437, NS-10489, and MH–27853, and an EMBO long-term fellowship to A,G.

Tetanic stimulation depresses the occurrence of Ca^{++} binding sites in synaptic vesicles of toadfish sonic muscle. ROBERT B. HANNA AND GEORGE D. PAPPAS.

The release of neurotransmitter at the neuromuscular junction is brought about by the influx of calcium ions into the presynaptic terminal following depolarization. It is therefore of importance to localize the calcium binding sites within the presynaptic terminal and to further elucidate their role in the release of acetylcholine. The sonic muscle of the toadfish (*Opsanus tau*) was selected for the investigation of calcium binding sites because it is capable of high frequency contractions (more than 200/sec) for periods of limited duration (Gainer and Klancher, 1965, *Comp. Biochem. Physiol.*, **15**: 159). The calcium binding sites can be visualized using the technique of Oschman and Wall (1972, *J. Cell Biol.*, **55**: 58). The calcium binding sites in the presynaptic terminal appear as single dense particles on the synaptic vesicle membrane. No calcium binding sites have been found on the presynaptic terminal membrane. Following stimulation of the sonic muscle for 10 min at 200 Hz, the calcium deposit on the synaptic vesicles membrane is no longer present. This was also true for a stimulation time of 1 min. Deposits are present as usual at other sites, such as mitochondria. In contrast to the frog neuromuscular junction (Pappas and Rose, 1976, *Brain Res.*, **103**: 362), the postsynaptic membrane in toadfish sonic muscle apparently did not show any increase of calcium uptake following tetanic stimulation. These results suggest that the calcium binding sites function to bind the calcium ions that enter the presynaptic terminal following depolarization and that the decrease in synaptic activity which follows tetanic stimulation may be the result of the inactivation of the calcium binding sites.

Inhibition of fertilization membrane elevation in sea urchin eggs by procaine and tetracaine. EMILY S. HARRIS, HERBERT SCHUEL AND WALTER TROLL.

The elevaton of the fertilization membrane in *Strongylocentrotus purpuratus* and *Arbacia punctulata* eggs at fertilization or upon stimulation by calcium ionophore A23187 was used as a bioassay to study inhibition of cortical granule secretion by two local anesthetics: procaine and tetracaine. The eggs were incubated in 10 mM procaine or 1 mM tetracaine for 10 min prior to fertilization. Fertilization membranes elevated from almost all the procaine treated eggs, but the perivitelline space was greatly reduced. A few of these eggs entered first cleavage without any sign of fertilization membranes. A greater percentage of tetracaine-treated *Arbacia* eggs appeared not to have elevated fertilization membranes. Procaine also reduced fertility and delayed cleavage in *Strongylocentrotus* eggs, while both drugs promoted polyspermy in *Arbacia* eggs. The effect of the local anesthetics was reversible by washing, but could not be overcome by raising the calcium concentration in sea water to 110 mM. Procaine and tetracaine impair, but do not prevent, cortical granule secretion in sea urchin eggs. The inhibition of fertilization membrane elevation may involve several mechanisms. Inhibition of protease action was not a factor since tetracaine did not change the activity released following fertilization. On the other hand, these tertiary amines can neutralize sulfated acid mucopolysaccharides secreted from the egg's cortical granules (Schuel *et al.*, 1974, *Exp. Cell Res.*, **88**: 24–30), as well as compete with calcium for membrane-binding sites necessary to trigger exocytosis.

Supported by grants from NSF (BMS 75–16126(and NIH (CA–15315).

Toadfish liver protein synthetic parameters at high doses of leucine in vivo. AUDREY E. V. HASCHEMEYER, MICHAEL A. K. SMITH AND RITA W. MATHEWS.

The effects of high chemical doses of an amino acid given in a single injection on protein synthetic parameters of toadfish liver have been examined. Anesthetized toadfish were injected *via* the hepatic portal vein with tracer or 15 mM ^{14}C-leucine. ^{3}H-mannitol was included to monitor the amount of injected fluid remaining in extracellular space. At the high leucine concentration, blood draining from the liver 1–2 minutes after injection shows a significantly higher ratio of leucine to mannitol (0.69) than at tracer dose (0.41). Leucine uptake into intracellular space (as fraction of dose) is markedly reduced; however, polypeptide chain assembly time (t_c), determined from the ratio of label in completed protein to total incorporation (completed protein + growing chains), is unchanged. At the 15 mM dose, $t_e = 4.8 \pm 0.5$ minutes ($N = 21$), compared to 4.6 ± 0.4 minutes ($N = 9$) in fish receiving tracer ^{14}C-leucine (temperature, 21° C). At 15 mM dose the proportion of uptake incorporated into protein was reduced consistent with a nearly two-fold expansion of the intracellular leucine pool. Comparison with actual uptake (35 nmoles/g liver) indicates that about 25% of liver intracellular space is initially occupied by the incoming amino acid. Nonuniformity of isotope distribution in the tissue may account for erroneous values for protein synthesis determined by other methods that depend upon values of whole tissue specific radioactivity. Attempts to determine liver protein synthetic rate by high dose (pool swamping) techniques at long incubation times have to date yielded low values for leucine incorporation into protein (*e.g.*, 5 nmoles/min/g) compared with fast kinetic methods (15 nmoles/min/g). Further exploration of high dose single injection and continuous infusion techniques is in progress.

Supported by NSF Grant BMS 75–10097 and NIH Grant HD-04670.

Structural and functional studies of the isohemoglobins from Fundulus heteroclitus. J. K. HAYNES, D. R. WILSON, M. PRICE, L. J. DANGOTT, P. MIED AND D. A. POWERS.

Fundulus heteroclitus erythrocytes contain at least four hemoglobin components that can be separated by ion exchange on DEAE cellulose. The separated isohemoglobins are pure according to starch gel and polyacrylamide gel electrophoresis. These isohemoglobins have tetrameric molecular weights of approximately 64,000 daltons. However, the dimeric dissociation constants, as measured by both sedimentation equilibria and large boundary gel filtration, are substantially different, suggesting subtle structural variations in the intersubunit contacts. Studies indicate that organic phosphates affect the oxygen equilibria and the subunit cooperativity of the hemoglobins. Furthermore, there are pH maxima for subunit cooperativity that are unique for each organic phosphate concentration. These data are especially significant in the light of our recent findings that organic phosphate and hematocrit are regulated in response to changes in environmental oxygen. *Fundulus heteroclitus* responds to hypoxia by first, increasing the amount of hemoglobin *via* increasing hematocrit; secondly, lowering organic phosphate, thereby increasing oxygen affinity in an oxygen-poor environment; thirdly, shifting the pH maxima of the subunit cooperativity; and fourthly, lowering blood pH to correspond with the other physiological variables. In the latter case blood pH is lowered by a rise in blood lactate due to an emphasis on glycolysis.

Supported by NSF grant GB–37548 to D. A. Powers and NIH grant GM–265 to the Physiology course at the Marine Biological Laboratory.

Preparation of tetramethylrhodamine-heavy meromyosin for actin localization by fluorescence microscopy. IRA M. HERMAN AND THOMAS D. POLLARD.

We prepared tetramethylrhodamine-heavy meromyosin (Rh-HMM) and demonstrated its ability to bind specifically to actin in glycerol-extracted myofibrils and cultured bovine endothelial cells. Tetramethylrhodamine-isothiocyanate at a concentration of 300 μg/ml was reacted for 18 hours with 12.4 mg/ml HMM at 0° C and pH 9.5. The Rh-HMM was purified from other reaction products by gel filtration on Sephadex G-25 and ion exchange chromatography on DEAE-cellulose. The mole ratio of rhodamine: HMM in the final product was 3: 1. Staining of glycerinated rabbit psoas muscle myofibrils was confined to the I bands. There was no fluorescence of the Z line or A band. The specificity of Rh-HMM binding to myofibrillar actin was shown in two ways. The staining by Rh-HMM is blocked by either preincubation with unlabeled HMM or by chemically inhibiting actin-myosin binding with 10 mM pyrophosphate. Cultured bovine endothelial cells were pretreated with 0.1% Triton X-100 and stained with Rh-HMM. There was intense fluorescence of stress fibers and a general, less intense fluorescence throughout the cytoplasmic matrix. The HMM-blocking and pyrophosphate-inhibition controls demonstrated the specificity of Rh-HMM binding to actin in these Triton-treated cultured endothelial cells.

Modulation of afferent activity of the posterior semicircular canal of the toadfish (Opsanus tau) *by efferent stimulation.* S. M. HIGHSTEIN AND ALBERTO L. POLITOFF.

Sagittally transected hemicrania with the labyrinth intact were placed in a recording chamber. Afferent activity of single posterior semicircular canal fibers was recorded, and the remainder of the nerve was placed in a suction electrode for stimulation. Afferent activity was sorted by a window discriminator generating a pulse out for each spike discriminated. These pulses were the input to a feedback circuit which controlled the pattern of stimulation, *i.e.*, delay and number of stimuli as well as the refractory period to further triggering. Spike autocorrelograms which also displayed the pattern of stimulation were generated as dot displays on an oscilloscope. Four patterns of response were observed: 1) pure inhibition, proportional to the number of stimuli (between one and four delivered at one per msec) lasting up to 200 msec; 2) reduction of the variability of spontaneous activity resulting in a greater regularity of discharge (this regularity of discharge often lasted for many seconds after the stimulation and gradually returned to control levels); 3) increase of the basic frequency of rhythmic activity (this effect depended upon the timing of the stimuli in relation to ongoing activity showing a clear refractory period); and 4) lack of effect.

Our results demonstrate separate effects first upon the absolute number of action potentials in a given time period and secondly upon the underlying frequency and regularity of discharge.

Dr. Highstein was supported by NIH 1R01 EY 01670–01, NIH I–K04 EY 00003–01, NIH 5 P01 NS–0751–08. Dr. Politoff was supported by NIH 1R01 NS–11588.

Comparative studies of plasma protein synthesis in temperate and Antarctic fishes. ALAN PAUL HUDSON.

In the toadfish, *Opsanus tau,* synthesis and secretion of plasma proteins were studied by means of hepatic portal vein injection of radioactively labelled L-leucine with subsequent monitoring of the appearance of label in acid-precipitable protein in the blood over time. Summer fish acclimated to 20° C show a lag time of 1.0 hr, associated with the secretory mechanism, and a half-time for accumulation of isotope in plasma protein of 1.1 hr. The plateau level of incorporation of this amino acid is reached about 6 hr after injection. In summer toadfish acclimated for two weeks to 10° C, the lag time is 6 hr, half-time for accumulation is 8 hr, and equilibrium for leucine incorporation is reached in about 24 hr. Winter toadfish acclimated to an ambient seawater temperature of 7° C show a lag time of 7 hr. The half-time for accumulation was somewhat greater, 12 hr, and equilibrium was reached at about 36–40 hr after injection.

In order to extend these studies to lower temperatures, a parallel series of experiments was carried out on *Dissostichus mawsoni* (the giant Antarctic "cod") at McMurdo Station in Antarctica. At ambient seawater temperatures of −1.5° C, the lag time before appearance of ^{14}C-leucine in completed proteins in the blood was 8 hr, and the half-time for accumulation was 20 hr. Plateau was reached after 60 hr. With ^{14}C-alanine as tracer, lag time for acid-precipitable plasma protein was 6 hr, half-time for accumulation was 10 hr, and equilibrium was reached at about 40 hr. Preliminary data were also obtained for synthesis and secretion of "antifreeze" plasma proteins. The results indicate a high degree of adaptation for protein metabolism in the Antarctic species. Complete kinetic analysis of the toadfish and *D. mawsoni* data is in progress.

Supported by NSF grants to Dr. A. E. V. Haschemeyer and Dr. A. L. DeVries.

Effect of 6-OH-dopamine, 5-OH-dopamine and adrenalin on teleost melanophore innervation and intracytoplasmatic microfilaments. L. C. U. JUNQUEIRA, R. ENOS AND F. REINACH.

Intraperitoneal injections of 6-OH-dopamine (80 mg/kg) promoted, in toadfish, killifish, summer flounder and winter flounder, a darkening of their color and loss of capacity to adapt to the color of the background. This condition persisted for three weeks after which the animals gradually returned to normal. The study of the skin of 6-OH-dopamine treated killifish showed degenerative lesions in the synapses of the melanophores and its fine nerves as early as 24 hours after the drug's administration. These lesions progressed and after four days no synaptic structures could be detected in these cells. This condition persisted up to the twentieth day. In melanophores of normal controls of the three above-mentioned species, a small number of microfilaments was observed mainly in the branches of these cells. These structures had a diameter of 11 nm. This was particularly evident in the toadfish and summer flounder. When these fishes were previously injected with the sympathomimetic drugs mentioned above, thick bundles of the 11 nm microfilaments appeared in the cell branches. Concomitantly their pigment granules migrated to the cell center. This effect was observed between 0.5 and 24 hours after the drug's administration. Pigment migration coincided with a constriction of the cell branches and swelling of the cell body. It is postulated as a working hypothesis that microfilaments induced by sympathomimetic compounds (known to produce pigment centripetal migration in teleosts) act by promoting the constriction of the cell branches and consequent pigment migration.

Orientation of catfish (Ictalurus nebulosus) *in strictly uniform electric fields: I. Sensitivity of response.* AD. J. KALMIJN, CLIFFORD A. KOLBA, AND VERA KALMIJN.

The aim of the present project was to obtain reliable data on the ability of catfish to orient with respect to uniform electric fields. In two circular fiberglass tanks, direct current fields were established by the use of multiple salt-bridge electrodes. A circular nylon screen separated the fish's habitat from the electrode area. In the animal's habitat the fields were uniform within 1%. At the beginning of each trial, the hiding tube in which the catfish resided was carefully moved to the middle of the tank, and the field was switched on. After a few minutes, the animal was deprived of its hiding place and taught to seek shelter in either one of two substitute tubes, depending upon the polarity of the field. The two substitute tubes were positioned along the nylon screen, 180° apart. One fish (A) was trained to swim toward the positive to reach the correct hiding tube. The other fish (B) was trained to swim perpendicular to the field lines, keeping the positive to its left. After an incorrect choice, the animals were chased to the opposite side of the tank; after a correct choice, they were left alone until the next trial. Great care was taken to eliminate and randomize any other orientational cues. Fields of 20, 10, 5, 2.5, and 1 μV/cm were applied. The resistivity of the water was kept at 2 kOhm-cm. The catfish could easily be trained to orient to fields down to 5 μV/cm, as had been found before. After prolonged training, the animals also learned to orient to fields of 2.5 and even 1 μV/cm. At 1 μV/cm, fish A made 82, and fish B 80 correct choices out of 120. The χ^2 values, calculated with the Yates correction for continuity, yielded for both cases probabilities of $P \ll 0.001$. The results clearly demonstrate that catfish can orient to strictly uniform DC fields of voltage gradients as low as 1 μV/cm.

This research originated from WHOI participation in the BUMP-MBL Behavior Course and was supported by the Office of Naval Research (Contract N00014-74-C0262 NR 083-004).

Orientation of catfish (Ictalurus nebulosus) *in strictly uniform electric fields: II. Spatial discrimination.* VERA KALMIJN, CLIFFORD A. KOLBA AND AD. J. KALMIJN.

In earlier experiments, we learned that catfish are able to discriminate between two PVC hiding tubes positioned along the periphery of a circular habitat, 180° apart, by the exclusive use of a uniform DC field. To establish how precisely the fish know to locate their shelter, we applied electric fields of 10 μV/cm and presented the animals with 4 PVC tubes (90° apart), 8 PVC tubes (45° apart), and eventually with 12 PVC tubes (30° apart). The experimental procedure was basically the same as described in the previous communication. In all cases, both fish scored a high number of correct choices. With 4 PVC tubes, fish A made 48, fish B 45 correct choices out of 60; with 8 PVC tubes, 41 and 42 out of 60; and with 12 PVC tubes, 76 and 88 out of 120. With 12 choices, the animals took considerably longer to decide upon the correct hiding place, but still did remarkably well. For the 12 PVC tubes, the mean directions ($\bar{\alpha}$) were off from the expected direction by only 3.56° and 1.11°; the lengths of the mean vectors (r) were 0.8409 and 0.8612, respectively. To test the statistical significance of the mean direction, we applied the modified Raleigh or V test, which yielded probability values of $P \ll 0.0005$. To determine whether the catfish made use of the distortions in the electric field around the PVC tubes, we presented fish B with 12 tubes made out of agar of the same resistivity (2 kOhm-cm) as the surrounding water. With the 12 agar tubes, we obtained without further training similar results as with the 12 PVC tubes. For the 12 agar tubes, we calculated: $\bar{\alpha} = 4.82°$, $r = 0.7654$, and $P \ll 0.0005$. In short, the animals are very well capable of selecting 1 out of 12 hiding tubes on the basis of strictly uniform DC fields.

This research originated from WHOI participation in the BUMP-MBL Behavior Course and was supported by the Office of Naval Research (Contract N00014-74-C0262 NR 083-004).

Gompertzian growth in cheilostome bryozoans. KARL W. KAUFMANN.

During a period of mid-summer to late fall the growth rates of three species of bryozoans, *Cribilina punctata, Schizoporella biaperta,* and *Hippoporina contracta,* were determined as a function of colony size. By counting the number of zooids at the beginning and end of time periods of two to four weeks, I could calculate the difference in the logarithms of the number of zooids divided by the length of the period. This gives an approximation of the specific growth rate, or the rate of growth of a colony per zooid already in the colony. Bryozoans increase in size by asexual budding of identical zooids, each with a lophophore for filtering phytoplankton out of the water column, and each with presumably the same metabolic requirements. One would expect that, for different sized colonies, the rate of growth per zooid would remain constant. It didn't. In fact, the specific growth rate decreased in direct proportion to the size of the colony.

Integrating the differential equation obtained from this linear relationship between specific growth rate and size yields a Gompertz curve. This is a sigmoid curve having an asymptote toward which the number of zooids converges. *C. punctata* was observed to fit the entire range of the curve up to the asymptote of about 300 zooids. The growth of *S. biaperta* and *H. contracta* was observed only over the lower parts of their curves, up to about 400 zooids. However, they had asymptotes of 3000 and 10,000 zooids, respectively.

The adherence to this simple growth pattern suggests that the colonies are acting as a whole, rather than as a collection of independent zooids, in regulating their growth.

Supported in part by NSF grant GB-30870 to T. Schopf and NSF grant DES72-01608 A01 to R. G. Johnson.

Demonstration of poly(adenosine diphosphate ribose) synthase activity during early sea urchin development and investigation of nicotinamide-induced cleavage arrest. S. S. KOIDE, R. P. ELLISON, L. O. BURZIO AND S. L. KOIDE.

Nuclei isolated from eggs and early embryos of the sea urchin, *Arbacia punctulata,* possess poly(adenosine diphosphate ribose) synthase activity. The synthase enzyme transfers the ADPR moiety of NAD^+ to various protein acceptors. Modification of nuclear structural proteins (histones) and enzymes (Ca^{++}, Mg^{++}-dependent endonuclease) by ADP-ribosylation has been demonstrated in a variety of organisms.

Arbacia synthase activity has a temperature optimum of 10–12° C and is inhibited by either 4 mM nicotinamide or 4 mM thymidine. Mg^{++} is required for activity of the egg nucleus enzyme. Isolated nuclei from unfertilized eggs, morulae (2-hour embryos) and prism gastrulae (17-hour embryos) have a low synthase activity relative to nuclei isolated from hatched blastulae (7.5-hour embryos). Nuclei of hatched blastulae, after 20 minutes incubation at 11° C, incorporated 111.9 pmoles $^3H\cdot NAD^+$/mg protein while nuclei of the unfertilized eggs, morulae and prism gastrulae incorporate 3.7, 19.2 and 11.3 pmoles $^3H\cdot NAD^+$/mg protein, respectively.

Nicotinamide completely blocks the cleavage of fertilized *Arbacia* eggs when added at 3, 10, 20 or 30 minutes after fertilization in concentrations greater than 25 mM. Fertilized eggs exposed to 4 mM nicotinamide for 10, 30, 60 and 120 minutes and washed five times with natural sea water resume cleavage. Washed nicotinamide-treated fertilized eggs can develop to the blastula stage, however 12–15% of the embryos develop abnormally. Nicotinic acid at

80 mM, L-glutamine at 68 mM and thymidine at 39 mM do not inhibit the cleavage of fertilized eggs or their development to the blastula stage.

Nicotinamide added to unhatched blastulae at concentrations greater than 5 mM prevents differentiation into plutei. Nicotinamide at a concentration of 4 mM blocks the incorporation of ^{3}H-thymidine into DNA of fertilized *Arbacia* eggs. These results suggest that the ability of nicotinamide to arrest cleavage is dependent upon its capacity to influence the poly(ADPR) synthase system.

This research was partially supported by NIH grant No. HD-05671.

Bioassay studies of acetylcholine in the stimulated frog sartorius nerve-muscle preparation and following denervation. M. E. KRIEBEL, D. R. MATTESON AND G. D. PAPPAS.

Paired sartorius muscles were placed in a chamber containing eserine and frog saline and mounted to the stage of a compound microscope. Small hooks were put into the fascia of one muscle on each side of a junctional region to minimize contractions. Miniature end-plate potential (MEPPs) were recorded from several cells to establish the mean of the major and small mode MEPPs (Kriebel and Gross, 1974, *J. Gen. Physiol.*, **64**: 85). The sartorius nerve was stimulated with a suction electrode at 10 Hz for 20–40 minutes. Strong contractions usually ceased after 5 min so that muscle cells could be repenetrated and the decrement in end-plate potential (EPP) followed. When the EPPs were reduced to 1/50 that of normal (or to S-MEPP size and failures), the preparations were dropped into homogenizing tubes containing 1 ml of boiling sea water for 1 min. Isolated clam hearts (*Mya*) were suspended in 1 ml of sea water (with eserine) with a kymograph lever. Hearts were sensitive to 10^{-9}–10^{-10} g/ml ACh, and only those which showed differences in tone and rate to ACh standard solutions of 20% increments were used. Tissue homogenates (1 μg ACh/g tissue) were bracketed with ACh standards. Some homogenates were incubated with eel AChE which destroyed all homogenate action. Mytolon also blocked the tissue ACh at the same concentration as needed in the standards. We found a slight increase (10%) in ACh after 15 min of 10 Hz stimulation and a 10% decrease ACh after 28 min. A sartorius nerve of several batches of frogs was sectioned and MEPPs disappeared at days 2 and 3 (the quiescent period) and reappeared at days 4 and 5 (Schwann cell MEPPs). During days 6–18 post nerve section, the ACh content was 50% of normal.

Acute thermal gradients as a possible cause of gas embolism and associated mortality in teleosts. RICHARD H. LAMBERTSEN.

Thermal stress in killifish, *Fundulus heteroclitus,* and the common eel, *Anguilla anguilla,* was studied. It was calculated that exposure to severe temperature changes within the normally tolerable range of these and other species would be sufficient to produce a 160 per cent saturation of their body tissues by nature of its diminishing effect on gas solubility in tissue. Such a state of supersaturation is of sufficient order of magnitude to provoke the formation of gas bubbles within the vasculature and tissues of the stressed fish. Microscopic examination of anesthetized fish exposed to sudden temperature changes (15–18° C/min) within their tolerable limits (6.0–36.0° C) provided evidence that bubble formation did occur. Bubble formation, however, was not extensive. Mortality rates were determined for *Fundulus* and *Anguilla* species over a three day period following exposure to severe temperature gradients (15–18° C/min) within their normal range of temperature tolerance. Experimental mortality rates did not differ significantly from control group mortality. Moreover, no increased mortality was observed when the experimental system was perfused with a nitrous oxide in oxygen mixture to enhance the ease of bubble formation. These results prompted the conclusion that although bubble formation may occur under condtions of extreme thermal gradients, it is not of sufficient severity to be acutely lethal in fishes within the size range of *Fundulus* or *Anguilla* species (8–80 g).

The formation of helical ribbons during in vitro *polymerization of tubulin from dogfish brain.* GEORGE M. LANGFORD.

Tubulin was extracted from dogfish brain and purified by two cycles of assembly-disassembly in 0.1 M MES buffer, pH 6.6, containing 1 mM EGTA, 0.5 mM $MgCl_2$ and 1 mM GTP.

Polyacrylamide gel electrophoresis of the purified tubulin samples showed that they contain very little high molecular weight proteins typically seen co-purifying with tubulin from mammalian sources. At 0° C purified tubulin existed almost exclusively in the form of 6S dimers as revealed by analytical ultracentrifugation. Samples negatively stained for electron microscopy corroborated this result showing that very few aggregates of any kind including ring or spiral structures were present. In some preparations, short, thin protofilamentous sheets were seen. After warming to 21° C for 30 seconds, large numbers of helical ribbons and flat sheets of protofilaments appeared. The coiled ribbons were present throughout the early stages of polymerization but not after polymerization was complete. Short microtubules were observed after 1 min at 21° C and grew in length during polymerization. Only microtubules were seen after polymerization was complete. The helical ribbons had an average width of 35 nm and contained approximately 8 protofilaments. The pitch angle of the helix was 33° and two or three turns were usually seen. The addition of 10 mM $CaCl_2$ to a polymerized or polymerizing solution of tubulin, converted all of the microtubules into helical ribbons. Some of the ribbons formed tubules when adjacent gyres of the spirals annealed. The diameter of these "macrotubules" ranged between 50 and 70 nm. The width of the Ca^{++} induced helical ribbons varied between two size ranges of 60 nm representing 11–13 protofilaments and 100 nm representing 19–21 protofilaments. The pitch angle of the spiral was 40°–45° and many turns of the helix were frequently seen. The presence of helical ribbons under normal polymerizing conditions raises the possibility that microtubules could form directly from the appropriate size helical ribbon. Apparently, excess Ca^{++} induces the formation of ribbons with incorrect numbers of protofilaments which form macrotubules instead.

Supported by the Josiah Macy Foundation.

Biological factors which effect the structure and functioning of salt marsh Aufwuchs *microfloral and meiofaunal assemblages.* John J. Lee, John H. Tietjen, Carmine Mastropaolo and Monica Lee.

Field and laboratory experimental studies were aimed at isolating and characterizing the biological factors which effect the successional trajectories and functioning of microfloral and meiofaunal assemblages important in coupling the lower and intermediate steps in the detrital food web of salt marshes. This summer we completed projects on the heterogeneous distribution of meiofauna and initiated several new ones.

Two distinctly separate approaches were used in the spatial heterogeneity experiments. Slides baited with particular species of algae and incubated in contact with the substrate in the greater Sippewissett salt marsh recruited more meiofauna than did those baited with other species. We interpret differences in the meiofauna captured on baited slides of the same alga but incubated at different sites in the marsh, or at different times, as a reflection of the match between the meiofauna available at the experimental site for recruitment and the algal species being tested at the time. In another approach we tested recruitment of meiofauna from natural collections and from gnotobiotic laboratory cultures to patches of different species of algae arrayed equidistantly in petri dishes. Selective recruitment of meiofauna was again demonstrated. One bacteriovorous nematode, *Rhabditis marina,* was repelled from patches of three algal species tested. We conclude that selective recruitment of meiofauna to particular algal patches could be an important factor in establishing the spacial heterogeneity so often observed in meiofaunal populations.

Two other experiments were incubated in the marsh. We observed the structural and functional changes which took place in the algal and meiofaunal populations during the summer in the presence and absence of different species of meiofauna caged or excluded from cages (1.0 m in area × 0.5 m high) in a large marsh tide pool. In another *in situ* experiment we studied the rates of *Spartina* detritus degradation by microorganisms alone and in the presence of meiofauna.

Supported by NSF grant GA-33388.

The action of osmium tetroxide on proteins and amino acids. J. C. Lisak, H. W. Kaufman, P. Maupin-Szamier and T. D. Pollard.

One-fourtieth the concentration of osmium tetroxide(OsO_4) routinely used to fix cells for electron microscopy rapidly and specifically cleaves actin and ovalbumin into peptides.

In the case of actin this leads to filament destruction. After a brief lag, viscosity of an actin filament solution is lost in a process having first order kinetics and a half time of about 30 minutes with 4 mM OsO_4 at 20° C in phosphate buffer at pH 7. The actin filaments are broken progressively into shorter and shorter fragments. About 30 molecules of osmium bind to an ovalbumin molecule. Serum albumin, insulin, and ribonuclease are not cleaved by osmium. Myoglobin, chymotrypsinogen and gamma-globulin are cross-linked, the latter two forming gels.

Spectrophotometric measurements at 365 nm showed that the relative reactivity of amino acids is cys > met > asn > arg > his > pro > all others > val. Representative amino acids showed an increased rate of product formation at higher pH. Changing the pH during a reaction showed that the reaction kinetics are determined by the initial pH. Two assays showed deamination of amino acids by osmium. First, all amino acids and a number of dipeptides reacted with osmium failed to react with ninhydrin stain. Secondly, all amino acids reacted with osmium failed to form dansyl derivatives. Dansylated amino acids, in the presence of excess osmium, formed a black product but remained unaltered as determined by polyamide thin layer chromatography. This suggests that several processes are occurring simultaneously at different rates. Para-amino benzoic acid also lost its reactivity with ninhydrin after reacting with osmium. We attempted to determine the n-terminal amino acids of the peptide fragments of actin and ovalbumin formed by osmium treatment. In both cases, no dansylated amino acids were identified. Our results indicate that the black product is due to both reduced osmium oxides $[OsO_2(H_2O)_n$, formed by oxidizing R-groups] and by coordination complexes.

Role of intracellular transglutaminase in the Ca^{2+}-mediated cross-linking of erythrocyte membrane proteins. LASZLO LORAND, LISA B. WEISSMANN, JOYCE BRUNER-LORAND AND DEBRA L. EPEL.

Transglutaminase activity in human erythrocytes, washed free of plasma and lysed by freezing-thawing, was measured at 37° C with the filter paper method of Lorand *et al.* (1972, *Anal. Biochem.*, **50**: 623) by incorporation of ^{14}C-putrescine into N,N′-dimethylcasein. In the red cell milieu as such, essentially no enzyme activity could be seen. The enzyme, however, was turned on to significant levels of activity by addition of small concentrations of Ca^{2+} ($\simeq 5 \times 10^{-4}$ M) as are known to accumulate in erythrocytes under certain conditions. Disc gel electrophoresis of the proteins of isolated cell membranes according to Steck and Yu (1973, *J. Supramol. Struct.*, 1: 220) revealed that exposure of lysates to Ca^{2+} ($5–25 \times 10^{-4}$ M, 0.5–6 hr, 37° C) caused formation of cross-linked polymers (X) which remained on top of the gels, while the 78,000 dalton 4.1 band disappeared. In the presence of trace concentrations of ^{14}C-putrescine (5×10^{-5} M) and Ca^{2+}, the isotope predominantly labels (X).

The Ca^{2+}-induced cross-linking pattern in cell membranes can also be obtained by treating (up to 6 hours at 37° C) energy-depleted cells with Ca^{2+} ($10–25 \times 10^{-4}$ M) or by incubating fresh cells with Ca^{2+} ($5–25 \times 10^{-4}$ M) in the presence of A23187 ionophore (Eli Lilly; 20–50 μg/ml in 1% dimethylsulfoxide). Histamine (*ca.* 10 mM) in the incubation medium prevents formation of (X).

The evidence suggests that cross-linking of proteins in red cell membranes, as that of fibrin during blood clotting (Lorand, 1972, *Ann. N. Y. Acad. Sci.*, 202: 6), ocurs through intermolecular γ-glutamyl-ε-lysine bridges and that the influex of Ca^{2+} (be it for metabolic reasons, senescence or sickling) into the cell causes polymerization by turning on the latent transglutaminase.

Aided by NIH.

Effects of the anti-viral agent Aphidicolin on early development of sea urchin embryos. MICHELE A. LORAND, SHELDON J. SEGAL AND SAMUEL S. KOIDE.

Aphidicolin, a tetracycline diterpenoid, isolated from *Cephalosporium aphidicola*, blocks early cleavage of fertilized eggs of the sea urchin, *Arbacia punctulata*, at concentrations of 2 μg/ml (7.36 μM) or greater. While a concentration of 1 μg/ml does not suppress early cleavage, the resultant blastomeres are irregular and the morulae which develop are abnormal and nonviable. Lower concentrations have no apparent effects on development through the pluteus stage. Concentrations of 2 μg to 20 μg per ml do not interfere with the subsequent transformation to plutei when added to cultures of pre-hatching blastulae. Thus, aphidicolin selectively inhibits early cleavage and does not prevent later cell divisions, morphogenetic movements, or cell differentiation. The zygotoxic effect observed with aphidicolin is not observed with similar or higher doses of the structurally-related antibiotic, tetracycline, or with chloramphenicol. At a concentration of 2 μg/ml, aphidicolin inhibits the incorporation of ^{3}H-thymidine into DNA of fertilized eggs. Other compounds that inhibit DNA synthesis, 5-iodo-2-deoxyuridine and cytosine arabinoside, do not inhibit early cleavage of fertilized eggs at 6-fold higher concentrations than the minimal effective dose of aphidicolin. The potency of aphidicolin as a zygotoxic agent and its specificity in affecting early cleavage at doses that do not cause abnormalities when introduced later in development suggests that the compound could, in mammals, interfere with early embryonic development without presenting a hazard to the maternal organism. This possibility, as well as the potential teratogenicity of aphidicolin will be tested in appropriate animal models.

The authors are grateful to Dr. S. Ikegami, University of Tokyo, for suggesting this study and providing a supply of aphidicolin. Supported in part by NIH grant HD-05671.

Studies on the organization and purification of sea urchin (L. pictus) *histone genes.*

ROBERTO MARCO, MIGUEL FLECHA AND LAURENCE H. KEDES.

While studying the accessibility to specific and nonspecific nucleases of actively transcribing chromatin in sea urchin, we have consistently detected a significantly lower number of histone genes in the DNA isolated from blastulae of *Lytechinus pictus.* The number of genes detected was 80 to 100 in contrast to the 460 genes known to be present in *L. pictus* sperm DNA. Two different assays have given the same result. The first measured the differences in the extent of hybridization to filter bound DNA of ^{3}H-labeled complementary RNA made *in vitro* by transcription of a cloned plasmid (pLp11) that contains a specific segment of *L. pictus* histone genes. The second compared the rate of reassociation of single copy *L. pictus* blastula DNA labeled *in vivo* with ^{3}H-thymidine to the rate of reassociation of the histone genes using as tracer the histone gene containing fragment labeled with ^{32}P by nick translation of the pLp11 plasmid. The most likely explanation for this apparent deamplification of the histone genes during development is that the actively transcribing histone genes are significantly more susceptible to degradation by endogenous endonucleases than the bulk of DNA. Consistent with this interpretation is our finding with the cRNA assay that the same number of histone genes are present in *Arbacia* plutei as in *Arbacia* sperm DNA. It is known that the rate of histone genes transcription, and thus possible nuclease accessibility is reduced at pluteus stages. Moreover, the size of nuclear DNA's isolated from these stages by gentle methods is fairly small (1 to 0.1 kb).

Using high molecular weight sperm DNA from *L. pictus,* we have been able to obtain significant enrichments in the histone gene containing DNA (up to 15-fold) by sucrose gradient isolation of the DNA after digestion with Bam I and Hind III restriction endonucleases, whose restriction sites are known to be absent from the histone gene repeat unit in this sea urchin species.

This work has been supported by NSF grant # PMC 76–09315. Roberto Marco acknowledges the support of Spanish Research Council and Miguel Flecha that of the Macys Foundation.

Diurnal variation in situ *of photosynthetic capacity and efficiency in* Ulva.

MICHAEL MISHKIND, DAVID MAUZERALL AND SAMUEL I. BEALE.

Ulva lactuca (sea lettuce) undergoes large diurnal oscillations of light saturated photosynthetic capacity *in situ.* Britz and Briggs have reported similar photosynthetic oscillations with accompanying variations in chloroplast orientation in cultured *Ulva.* Freshly collected samples of lightly pigmented *Ulva* from Great Harbor, Woods Hole, Massachusetts, had a maximum white light saturated (36 mW/cm^2) rate of 540 μmoles O$_2$/hr/g fresh weight, and a minimum of 140, at noon and midnight, respectively. However, at low light intensity (2 mW/cm^2), the rate of O$_2$ evolution remained constant (90 ± 10 μmoles O$_2$/hr/g fresh weight) throughout the diurnal cycle. Samples kept in continuous light ($2 mW/cm^2$) maintained a similar oscillation of photosynthetic capacity for at least 36 hr, the amplitude gradually decreasing. After two weeks of continuous illumination, the photosynthetic capacity was constant (320 μmoles/hr/g fresh weight) and near the midpoint of the oscillations. Darkly pigmented samples of *Ulva* (four times more chlorophyll), freshly collected from a shaded habitat, had similar oscillations of the light saturated rate. At low light intensities the rate of O_2 production is proportional to the photosynthetic quantum yield, the light absorbed, and the number of photosynthetic units. Our data indicate that these factors remain constant over the diurnal cycle. Light saturation occurs when the absorption rate exceeds the slowest dark reaction rate. The overall rate at light saturation is then proportional to the slowest dark reaction rate. Our data show large variations in the light saturated rate, thus a change in the dark reaction rate is responsible for the diurnal oscillation. These was only a rough correlation between chloroplast position and photosynthetic capacity. During the face-to-edge movement of the chloroplasts in the lightly pigmented cells, the *in vivo* light absorption at 680 nm remained at 37 ± 8%. This small effect would change the low light rate of photosynthesis, but not the light saturated rate, thus indicating that the chloroplast movement *per se* is not the cause of the large diurnal oscillations. The assigned cause, *i.e.*, the variation in some limiting dark reaction, may be related to a diurnal reassignment of metabolic priorities, which in turn could be coupled to cell division and chloroplast movement.

Effects of metabolic inhibitors on the electrical properties of lobster muscle fibers. WILLIAM MOODY JR.

Dicumarol, an uncoupler of oxidative phosphorylation, causes a dramatic increase in the electrical excitability of slow flexor muscle fibers of the lobster (*H. americanus*). Normally these fibers do not generate action potentials; they show small (<10 mV), graded responses to depolarizing currents. After exposure to dicumarol (2×10^{-5} M) for 2.5 hr at 30° C, approximately 85% of the fibers generate full, all-or-none action potentials when depolarized. This effect occurs with a delay of ~ 30 min after addition of the drug and proceeds gradually thereafter, the fibers showing a continuum of states of increasing responsiveness to depolarization. These intermediate stages take the form of repetitive oscillations of membrane potential which occur above a definite threshold depolarization. The entire transition in electrical properties has been followed in single fibers. The effect of dicumarol occurs without a consistent change in the resting potential or input resistance of the fibers; some fibers show small contractions concurrent with the action potentials. Experiments on the homologous muscle in crayfish (*P. clarkii*) have shown that the same increase in electrical excitability is caused by three uncouplers: dicumarol (10^{-5} M), DNP (2×10^{-4} M), and carbonyl-*m*-chlorophenylhydrazone (10^{-6} M). The time course of the dicumarol effect in crayfish is strongly temperature-dependent, is greatly accelerated by cyanide (2 mM), and is markedly slowed by iodoacetate (10^{-4} M), an inhibitor of glycolysis. The hypothesis that this action of uncouplers is secondary to their metabolic effects, and is due to accelerated lactate production and fall in internal pH is being considered.

The author was a Grass Fellow.

One of the consequences of being a diatom: a simulation analysis of the reproductive strategy of diatoms. JOHN F. MURATORE.

It is a peculiarity of some diatoms that in asexual division two daughter cells are produced, one of which is the same size as the mother cell and one which is one size class smaller. It is interesting to analyze the effects of this behavior on the mean size of the population through the technique of computer simulation. A model of this behavior which assumes synchronous reproduction was developed in APL. The model showed that the population will always distribute itself so as to form a distribution with constant proportions between size classes. Thus the system will always go to a constant mean size. Increasing the number of size classes simply increases the constant mean size achieved. Changing the starting population simply affects the time necessary for the system to achieve this constant value. A model which assumes that diatoms reproduce asynchronously was also developed. This assumes that for each size class there is a certain amount of time between the entry of a cell into that size class and its reproduction. Thus the model consists of different intervals between birth and reproduction for various size classes. The model is also adapted to allow various members of a size class to be in different states of preparation for reproduction at the start of the simulation. This model shows that after a period of initial oscillation the mean size goes to a constant value. This constant mean size was observed in cultures of *Nitzschia* sp. by Wiedling (1948, *Bot. Notsier.*, **1948**: 322 354). Various types of curves he observed for mean size are easily generated by the model. This constant size hypothesis would indicate that there is a constant amount of diatom biomass per unit volume at a given time in the population. Thus, the maximum energy gained by a nonsize selective zooplankter in this system is a function of density. Under certain conditions, it might be thus energetically unfeasible for a zooplankter to graze on a patch of diatoms. Finally, the model generated the degree of variance observed by Wimpenny (1944, *J. Mar. Biol. Ass. U.K.*, **26**: 271–284) in *Rhizosolenia* sizes in the North Sea.

Toadfish liver elongation factor 1: time course of cold acclimation and hormonal effects. Jennifer B. K. Nielsen.

Measurements of the specific activity and molecular weight distribution of elongation factor 1 (EF-1) in the post-ribosomal supernatant of toadfish liver at summer temperatures of 20° C and following two weeks of acclimation at 10° C have been made using a poly(U) dependent polymerization assay. Extensively salt-washed ribosomes from rat liver were used and supplemented with excess toadfish liver elongation factor 2. The specific activity of EF rises from a 20° C acclimated level of 7.7 ± 1.6 (16 fish) to 13.7 ± 2.0 (16 fish) pmole/min/mg post-ribosomal supernatant protein following two weeks at 10° C. EF-1 from 20° C acclimated fish is about equally distributed between forms with a molecular weight of 400–600,000 daltons (heavy) and forms of 50–200,000 daltons (light). Acclimation produces an increase exclusively in the lower molecular weight forms, resulting in a 30% heavy, 70% light distribution in cold acclimated fish. This response does not appear until 10 days after transfer to 10° C. There is then an increase in the lower molecular weight species to reach the new steady state by 14 days. In order to determine what triggers the increase in specific activity of the light forms of EF-1, preliminary experiments have been carried out on the effects of several hormonal treatments on polypeptide chain elongation. Daily injections of hydrocortisone (100 μg/100 g) for three days, of triiodothyronine (40 μg/100 g) for three days and of triiodothyronine every two days for eight days did not change the elongation rate measured *in vivo* or levels of EF-1 in post-ribosomal supernatants.

Supported by NIH Grant HD 04670 to Dr. A. Haschemeyer.

Somatostatin biosynthesis in anglerfish islets. Bryan D. Noe, Gordon C. Weir and G. Eric Bauer.

Somatostatin (SRIF) has been localized in cell bodies of hypothalamic nuclei, gastric and intestinal mucosae, and in D-cells of pancreatic islets from mammals, birds, and fish.

Abundant SRIF determined by immunohistochemical means in anglerfish islets prompted us to examine this tissue for SRIF synthesis. After isolated anglerfish islets were incubated with ^{3}H-tryptophan (Trp), ^{14}C-isoleucine (Ile) or ^{35}S-cystine (Cys) for various time periods, proteins were extracted in 2 M acetic acid and desalted by Bio-Gel P-2 gel filtration.

Proteins present in the void volume were separated by P-10 gel filtration and then isolated by 10% polyacrylamide gel electrophoresis at pH 9.5. The major immunoassayable SRIF of the extracts eluted just prior to the salt region on P-10 gel filtration, and migrated slowly toward the cathode on electrophoresis. The behavior of synthetic ovine SRIF was the same. During pulse incubations, the incorporation of ^{3}H-Trp and ^{35}S-Cys into SRIF increased with time (after a lag of *ca.* 60 min) while no ^{14}C-Ile appeared in the SRIF region. Cycloheximide (100 μg/ml) totally inhibited labeling of anglerfish SRIF. Since mammalian SRIF contains no Ile, but contains one Trp and two Cys residues, the results of isotope incorporation studies suggest that anglerfish SRIF also is devoid of Ile, but contains Trp and Cys. (Anglerfish insulin and proinsulin contain Ile and Cys, but no Trp, while anglerfish glucagon and proglucagon contain Trp but no Ile or Cys.) When islets were subjected to short incubations with labeled Trp and Cys (pulse) followed by incubations in the absence of isotopes (chase), the incorporation of Trp and Cys into SRIF increased with the length of chase, suggesting the involvement of a larger precursor in SRIF synthesis. Work on the identity of a SRIF precursor, and the chemical and biological characteristics of anglerfish SRIF are in progress.

Electrical activity and structure of receptor and second-order cells of the median ocellus of the dragonfly. John A. Patterson and Richard L. Chappell.

Cells producing a response, described as typical of the second-order ocellar neuron response, have been identified by procion yellow staining *via* intracellular micropipette recording electrodes. Of those cells successfully filled, two have been unequivocally identified as representing the same second-order neuron on the basis of gross anatomy, location, and patterns of terminal branches in the brain. The latter has been confirmed by comparison with a catalog of the central projections of the seven large neurons in the dragonfly median ocellar nerve obtained using cobalt impregnation of the whole nerve. The identified neurons represent one of three pairs of neurons, a neuron from each pair terminating in one side of the brain. (The seventh large ocellar neuron sends branches into both sides of the brain). The procion study reveals that the identified neuron descends from one lobe of the bilobed median ocellar retina, crossing over in the ocellar nerve to terminate in the contralateral deutocerebrum, an observation difficult to confirm from cobalt impregnations alone.

Procion staining of three receptor cells of the dragonfly median ocellus using intracellular electrodes has always revealed the entire receptor axon as well as the retinular region and soma. Receptor axons may be straight or slightly serpentine in their course and show no major branches, but have small swellings along their length. In each case the receptor axons terminate in the synaptic region of the retina and do not send projections into the brain. It may well be that the fairly numerous small axons in the ocellar nerve represent a class of second-order cell which may not be fully characterized electrophysiologically.

This work supported by the award of a Research Associateship of the Graduate School of the City University of New York (to JAP), and by NIH Grant EY–00777 and CUNY FRAP Grant 11059 (to RLC).

Echinochrome oxidation in Arbacia punctulata *embryos.* George Perry.

Early after fertilization, the free calcium ion concentration in *Arbacia* embryos increases. Calcium increase may be responsible for the respiratory burst following fertilization, since exogenous calcium added to *Arbacia* egg homogenates induces a large oxygen consumption. The homogenate O_2 consumption is KCN insensitive, as is approximately one-half of the respiration occurring in the whole fertilized egg. The cyanide insensitive component is calcium controlled and is not an artifact of cyanide oxidation. The reported result of cyanide causing eggs in which the respiration had been stopped by heating to 50° C for five minutes to resume O_2 consumption could not be reproduced.

In the homogenate, oxygen consumption can be explained by Ca^{++} action on echinochrome. Calcium in the millimolar range binds to echinochrome in a catalytic manner, increasing echinochrome oxidation several hundred-fold at intracellular pH. Yet at pH above 8, oxidation occurs spontaneously. The pigment granules in which echinochrome is compartmentalized are very acidic, probably less than pH 5, and release this acid upon lysis with triton X-100 or calcium. *In vitro* echinochrome does not interact with calcium at this pH to consume oxygen.

Upon calcium binding, there is a vast decrease in absorbance at 475 nm, not dependent on O_2. Another more gradual color change occurs that is dependent on oxygen.

One mole of crystalline echinochrome reacts with one mole of O_2. In unfertilized egg homogenates one-half mole of oxygen is consumed for each mole of echinochrome present. This could either be because the echinochrome is half oxidized in the cell, reacts differently when attached to its protein or is producing H_2O_2 which subsequently breaks down due to endogenous catalase activity.

The redox potential measured by dropping mercury polarigraphy was +0.05 volts at pH 7.0, a value consistent with an electron transport function for echinochrome.

Temperature dependency of leucine transport by toadfish liver in vivo. Roger Persell.

The effect of acute reduction of body temperature from 21° C to 10° C on L-leucine transport *in vivo* was examined in the liver of the toadfish (*Opsanus tau*). Characterization of the amino acid transport system in liver is based upon the rapid uptake of ^{14}C-amino acid injected into the hepatic portal vein relative to ^{3}H-mannitol supplied as an extracellular space marker. The time course of disappearance of mannitol, which is dependent on blood flow, shows a slight temperature effect ($Q_{10} = 1.3$) in this range. Uptake of ^{14}C-leucine into intracellular space is strongly temperature-dependent with uptake rate constant decreasing from 2.7/min at 21° C to 0.7/min at 10° C ($Q_{10} = 3.8$). The rate constant for efflux from intracellular space decreases from 1.1/min to 0.4/min ($Q_{10} = 2.8$). Incorporation of labeled amino acid into protein exhibits a strong temperature dependency ($Q_{10} = 10$). Average polypeptide translation time increases from 4.8 min at 21° C to 20 min at 10° C ($Q_{10} = 3.8$). The magnitude of the temperature effect on amino acid incorporation suggests a decrease in number of active ribosomes in addition to the slowed elongation rate. Kinetic analysis of the time course of leucine uptake and incorporation into protein at the two temperatures is consistent with a carrier-mediated transport process, characterized by 40% uphill or active transport at 21° C and 20% active transport at 10° C.

Supported by NSF Grant GB–42752 to Dr. A. Haschemeyer.

Comparative respiration of Cape Cod ecosystems. Bruce J. Peterson, Charles A. S. Hall, James P. Reed and Tim Wood.

The dark respiration of five local terrestrial and aquatic ecosystems was measured during summer 1976. Estimates were based upon measured changes in O_2 concentration in dark bottles (marine plankton), *in situ* chambers (marine benthos, *Fucus* beds and salt marshes) and water (freshwater stream). Forest respiration was determined by changes in CO_2 in *in situ* chambers. All rates are reported as mg $O_2/m^2/hr$.

Respiration at Hadley Harbor totaled 115 mg $O_2/m^2/hr$, including the marine plankton in the 5 m water column (100) and the benthos of the mud bottom (15). The rate for the *Fucus* bed at Quisset Point was 930, of which the *Fucus* community accounted for 810 and invertebrates (primarily *Littorina*) 120. Respiration of the North River tidal marsh combined estimates for *Spartina alterniflora* plus epibenthic algae (1020), waters of a tidal creek (360), mudflats (250) and marsh pannes (160) for a weighted average of 800 $O_2/m^2/hr$. Rates of respiration in the *Elodea*-rich Coonamesset River were about 800 mg $O_2/m^2/hr$ attributable principally to the *Elodea*, since plankton respiration was undetectable. Respiration in the Quisset oak forest totaled 1070 mg $O_2/m^2/hr$, including the forest floor plus shrub layer (450), stems and branches (270) and foliage (350).

Ecosystem dark respiration rates were remarkably similar (800–1100 mg $O_2/m^2/hr$) in both aquatic and terrestrial sites where vegetation was abundant. The marine community had a lower respiratory rate of about one-tenth that in the other sites.

The authors thank the staff and students of the MBL summer ecology course for their assistance in the field. Special thanks are due John Day, Ed Lemon, Ken Mann, Ted Packard and Mario Pamatmat for technical guidance.

Regional localization of translatable RNA in Xenopus laevis. Carey Phillips, Joan Ruderman and Mike Rosbash.

This work has addressed the question of the localization of macromolecules which may potentially shape the developmental course of regional areas within the developing embryo. As a first approach to this question, RNA was isolated from three regions of the *Xenopus laevis* embryo at beginning gastrula. *Xenopus laevis* embryos were chosen as the source of experimental material because of their large size, ability to orient in an animal-vegital axis following fertilization and the large amounts of synchronous material which can be obtained. *Xenopus* eggs contain approximately 4 μg of total RNA per egg of which approximately 4 ng per egg represents messinger RNA which has been synthesized and accumulated during oogenesis. A question which can be asked of this system is whether maternally derived RNA is regionally localized.

Stage-9 embryos were dejellied, oriented in the same plain and animal-vegital axis and frozen in water. The embryos were sectioned into three regions in a cryostat and RNA was isolated from each region. The RNA was translated in the wheat germ *in vitro* translation system in the presence of ^{35}S-met. The translation products were analyzed on O'Farrell 2-dimensional gels and exposed to X-ray film. Preliminary results suggest that there are about eight quantitative differences in the small, basic to neutral proteins found in the animal and vegital regions.

We would like to thank Gerry Krystal for his expert technical advice. This work was supported by NSF grant #PCM 76–09315. C.P. is supported by an NIH predoctorial training grant. J.R. is a Jane Coffin Childs post-doctorial fellow.

Isethionate blocks release of transmitter from isolated secretory vesicles and from frog neuromuscular junction. HARVEY B. POLLARD, ANTOINETTE STEINACKER AND CHRISTOPHER PAZOLES.

The chemistry of release from isolated secretory vesicles is widely studied in hopes of gaining a molecular perspective on the mechanism of secretion from cells. Release from isolated adrenergic secretory vesicles (bovine chromaffin granules) is blocked by isethionate. (Isethionate naturally occurs in squid axoplasm at a concentration of 160 mM.) Exposure of the vesicle to Ca^{++}, Mg^{++} and ATP allows extravesicular chloride to enter the vesicle through an anion channel and thereby generate an osmotic imbalance and subsequent release. Isethionate blocks release by blocking the vesicle anion channel. We were thus interested in whether anion channels were also involved in release from cells, and we tested the frog neuromuscular junction. We report that when isethionate replaced 98% of the chloride, miniature end-plate potential (MEPP) frequency declined to nearly zero. This effect of isethionate was dose dependent: near maximal inhibition was obtained when isethionate replaced 50% of the chloride and replacement of 25% of the Cl produced measurable inhibition. With 50% replacement, evoked end-plate potential (EPP) amplitude was reduced by more than 70%. We concluded that the effect of isethionate was presynaptic since quantal content of EPPs fell substantially, while MEPP amplitude remained constant. Blockage of transmission could be reversed by 6-fold increase in Ca^{++} (3.6 mM) in the 50% isethionate medium. By contrast, isethionate itself was found to have no affinity for calcium, as measured by the arsenazo-III method. In conclusion, isethionate is capable of producing a presynaptic inhibition at the neuromuscular junction which is calcium dependent. This finding may prove to be of general significance, in as much as synaptic transmission and pre-synaptic inward Ca^{++} current in the squid stellate ganglion were also blocked by isethionate (R. Llinas and K. Walton, personal communication). The relation of the mechanism of action of the inhibition of presynaptic release to the action of isethionate on vesicles is as yet unclear.

Maximally-fast measurement of complex admittance of squid axon through digital Fourier transform. D. POUSSART, L. E. MOORE AND H. M. FISHMAN.

The small-signal admittance provides a complementary probe to the study of ionic and charge movements and their kinetics, in excitable membrane. Measurements have been restricted to near rest potential because available methods have required lengthy data acquisition. Squid axon, under axial wire, does not survive prolonged polarizing currents well. An efficient method, with speed near the theoretical limit for time-frequency domain analysis, has been developed. A 1 mV peak-to-peak pseudo-random binary sequence (PRBS) is superimposed as a small perturbation on a normal step clamp potential. The response is sampled and processed by digital Fourier transform (DFT) while maintaining precise register between the PRBS and the sampling through a form of time compression-expansion. A PRBS has a white magnitude spectrum (with a small band edge correction), hence the DFT of the response yields a calibrated magnitude plot of admittance directly. The phase spectrum of a PRBS, however, has a complex, but constant, form. This reference quantity is automatically subtracted, thus providing absolute phase data. All computations within this instrument (Patent pending, Poussart and Ganguly) are carried out digitally in 70 msec for 256 frequency components. Measurements were performed between 0.1 and 2560 Hz, the latter requiring a perturbation as short as 100 msec. As a result of high speed, potentials from −90 to beyond +30 mV could be explored. In external sea water, the interaction of K and Na conductances is reflected as a pronounced anti-resonance. This feature is considerably modified by external TTX (1 μM) application. Internal perfusion with 0.29 M CsF virtually reveals the Na conduction alone, with a low-frequency phase which increases beyond 180° in correlation with steady-state inward current condition. These and other related data provide a detailed, linearized description of the various conduction processes. The method has wide applicability to studies of other time-varying systems.

Aided by CNRC grant A5274, and NIH grants NS-80409 and NS-11764.

Cyclic contraction and relaxation of glycerinated myofibrils isolated from skeletal muscle. M. M. PRATT, M. S. MOOSEKER, D. P. KIEHART AND R. E. STEPHENS.

We have observed cyclic contraction and relaxation of sarcomeres in isolated myofibrils from glycerinated skeletal muscle. Myofibrils 20–100 μm in length were prepared by homogenization of glycerinated rabbit psoas muscle fibers in 75 mM KCl, 2 mM $MgSO_4$, 1 mM DTT, 0.5 mM ATP, 0.05 mM EGTA, 10 mM potassium phosphate, pH 7.0. The muscle had been glycerinated for over eight months in 50% glycerol, 0.1 M KCl, 10 mM Tris-maleate buffer, pH 7.0. Cyclic contraction and relaxation was observed in these myofibrils after addition of contraction solutions containing 75 mM KCl, 2 mM $MgSO_4$, 0.2 mM DTT, 0.5 mM ATP, 5 mM EGTA, 10 mM potassium phosphate, pH 7.0, to which $CaCl_2$ was added to give free Ca^{++} ion concentrations of 10^{-7} to 10^{-9} M. Ca^{++} ion concentration was estimated by the method of Portzehl, *et. al.* (1964, *Biochim. Biophys. Acta*, **79**: 581). One-shot contraction, typical of isolated myofibrils, was observed at concentrations of Ca^{++} above 10^{-6} M or at temperatures above 27° C. Observations were made using phase contrast microscopy and were documented cinematographically. Two general classes of contractile behavior were seen: first, cyclic contraction and relaxation of sarcomeres that showed little coordination between adjacent sarcomeres; and secondly, wave-like propagation of contractions along the length of myofibrils. In both kinds of cyclic behavior, a single contraction and relaxation of a sarcomere usually occurred with a frequency of 1–2 cycles per second. Pulsating myofibrils were almost always attached to the slide or the coverslip suggesting that the tensile force for relaxation was provided by passive stretch. One observation of cyclic contraction in a free floating myofibril, however, may suggest the existence of an elastic component within the myofibril.

Supported by NIH grant GM 265 to the Physiology course, MBL.

The incorporation of an exogenous ATP-ase (apyrase) into the synaptic vesicles of the frog sartorius neuromuscular junction causes block of synaptic transmission and vesicle depletion. A. PREVITE, S. ROSE, A. BLITZ AND A. L. POLITOFF.

Adenosine triphosphate (ATP) is known to be present in synpatic vesicles and to be released presynaptically, in association with the transmitter substance. In 7 experiments, the preparations were exposed to Ringer's containing up to 4 mg/ml of apyrase (an ATP-ase, 3 units/mg, Sigma) and the end-plate potentials (EPP's) were recorded at 15 min intervals during several hours, before and after apyrase. At this low rate of stimulation no change of EPP's amplitude was noticed. In contrast, stimulation at 12 Hz for 25 min followed by a rest period of 45 min ("incorporation procedure") abolished or greatly reduced the EPP's of preparations treated with apyrase (0.1–1.0 mg/ml, "experimentals") but did not change the EPP's of control preparations treated with equal amounts of thermally inactivated apyrase (75° C, 25 min). In 3 out 11 experiments the incorporation procedure had to be repeated two times (2 experiments) or three times (1 experiment) in order to show block of the EPP's of experimentals. After such block, specimens were fixed and reacted for histochemical demonstration of ATP-ase activity (lead method) in the presence of ouabain and absence of magnesium. After block of EPP's, the experimentals showed reaction product, mostly inside the few remaining synaptic vesicles. Some terminals had total vesicle depletion and reaction product was seen free in the terminal. Preparations not treated with active ATPase did not show vesicle depletion. Intravesicular ATP may be needed for the formation of new vesicles and/or reduce release of transmitter.

Supported by NIH grant 1 R01 NS-11588.

Evidence for in vivo *bioradiation at wavelengths greater than 6400 Å in* Gonyaulax polyedra. GEO. T. REYNOLDS.

Sweeney and Hastings have published action spectra for phase shifting, photoinhibition, and photoenhancement in *Gonyaulax* (Sweeney, Haxo and Hastings, 1959, *J. Gen. Physiol.*, **43**: 285–299; and Hastings and Sweeney, 1960, *J. Gen. Physiol.*, **43**: 697–706); and peaks appear in these action spectra in two regions: one between 4000 Å and 5000 Å and another between 6000 Å and 7000 Å. Using a diffraction grating spectroscope-image intensifier system previously described (Reynolds, 1975, *Biol. Bull.*, **149**: 443), sensitive from 4000 Å to 6200 Å, the luminescence peak is observed at 4725 Å, and the light output becomes immeasurably small or zero at 5800 Å. The spectroscope image intensifier system is not sensitive at wavelengths greater than 6200 Å and the dark adapted human eye is 3 orders of magnitude below its maximum sensitivity at 6400 Å. To investigate this long wavelength region a photomultiplier with an extended red sensitivity cathode was used, in combination with selected barrier and narrow band pass filters, which resulted in sensitivities in wave length intervals 6400 Å to 9000 Å, 6300 Å to 6500 Å, 6500 Å to 6700 Å 6700 Å to 6900 Å, and 7400 Å to 7600 Å. Significant radiation was found to be emitted *in vivo* between 6300 Å and 6900 Å, with the suggestion of a peak in the region 6400 Å to 6600 Å. No detectable radiation was found at 7500 Å.

Expression of differentiated function in cultures of isolated sea urchin micromeres. ALLEN J. ROSENSPIRE, THEODORE A. BREMNER AND BURTON M. POGELL.

Blastomeres from 16-cell stage embryos of *Arbacia punctulata* were reproducibly separated into three cell fractions by the following modifications of the procedures of Spiegel and Spiegel (1975, *Amer. Zool*, **15**: 583–606). First, fertilization membranes were removed by treatment for ten minutes with 0.04% papain-4 mM DTT (pH 8). Secondly, individual cells were released at the 16-cell stage by passage through a capillary-tipped disposable pipette. Finally, micromeres, mesomeres, and macromeres were separated by settling through a linear isosmotic 0.05 M–0.5 M sucrose gradient at $1 \times g$ and 0° C.

The formation of large numbers of spicules by micromeres after aggregation and cell division in the presence of 2% horse serum-sea water, as described by Okazaki (1975, *Amer. Zool.* **15**: 567–581), was confirmed in five different preparations. First detection of spicules, as revealed by birefringence in a polarized microscope, occurred after 20–36 hours of culture at 25° C. Spicule formation, but not aggregation and cell division, was completely inhibited by actinomycin D (0.7 μg/ml) and markedly reduced in time of appearance and both number and size by a whole sonicate of mesomeres. However, the presence of bromodeoxyuridine (3 μg/ml) had no effect on the expression of micromere differentiated function. No spicule formation occurred in cultures of isolated mesomeres or macromeres ± horse serum, although aggregation was enhanced by serum addition. Also, the addition of sonicated extracts of micromeres to mesomeres did not cause any appearance of spicules. These results confirm the determination of differentiation in micromeres at this early stage of sea urchin development and the possibility of using quantitative assays of collagen and $CaCO_3$ formaton in spicules as a model system for assay of cytoplasmic factors involved in specific gene expression.

This work was supported by grant # PCM 76–09315 from the National Science Foundation.

Regenerative activity in the presynaptic terminal region of the barnacle photoreceptor. W. N. ROSS AND A. E. STUART.

We have studied with intracellular recording the membrane properties of the terminal region of the median photoreceptors of the giant barnacle, *Balanus nubilus*. These four large receptors respond to light with an initial depolarizing transient followed by a steady plateau.

Following illumination, the membrane hyperpolarizes beyond dark resting potental and slowly returns to rest. Both the depolarizing and hyperpolarizing responses are conducted electrotonically with high fidelity from the somata of the receptors, located in a peripheral ocellus, to their terminals in the supraesophagael ganglion often 10 mm or more away.

In the presynaptic terminal region the transient part of the receptor potential causes a small regenerative depolarization which can, on occasion, appear as a larger action potential of variable amplitude. 10^{-6} M tetrodotoxin has no effect on this terminal action potential or on the receptor potential, although it blocks impulses in ganglion cells. Replacing sodium with choline also has no effect on the spike. However, 30 mM cobalt blocks the action potential reversibly. When the light response is below threshold for the regenerative event, the action potential can be elicited by bathing the terminal region in 6 mM TEA, 10 mM procaine, or 20 mM barium instead of calcium. The amplitude of the action potential elicited with TEA depends on the external calcium concentration and is abolished when calcium is removed. These pharmacological effects are consistent with a calcium-dependent regenerative mechanism. Simultaneous recordings from one receptor axon with two separated microelectrodes indicate that the action potential is generated locally in the terminal region and conducted backwards electrotonically. Similarly, those pharmacological agents which cause a spike in the terminal region do not do so when applied to the axon region of the receptors. This regenerative event may play a role in synaptic transmission, or may be a manifestation of a mechanism which amplifies the light response.

Supported in part by U.S.P.H.S. Grant EY-01188.

Evidence for in vivo *bioradiation at wavelengths greater than 6400 Å in five strains of luminous bacteria.* E. G. Ruby, Geo. T. Reynolds, A. J. Walton, and C. J. Hardy.

A diffraction grating spectroscope-image intensifier system, previously described, and an extended red sensitivity cathode photomultiplier described in an accompanying paper (Reynolds, 1976, *Biol. Bull.*, **151**: 427) have been used to investigate the spectral distribution of light emitted *in vivo* in selected luminous bacteria. By means of the spectroscope-image intensifier system, the visible spectrum between 4000 Å and 6200 Å was recorded. By means of the photomultiplier, in combination with selected barrier and interference filters, the spectral region between 6300 Å and 9000 Å was investigated. In all five strains of bacteria investigated, significant radiation was found between 6300 Å and 7500 Å.

In *P. phosphoreum, P. harveyi, P. leiognathi,* and *P. fischeri* peaks were found in the visible spectrum in the neighborhood of 4800 Å to 5100 Å. The amount of radiation at wavelengths longer than the visible varied among the species, in the range of several per cent of the total visible radiatioh. This radiation can be accounted for by an extension of the visible spectra, which are still nonzero at 6200 Å, to a vanishingly small emission at 7500 Å.

Of particular interest was the bacterium *P. fischeri* Y1, which has a yellow luminescence, rather than the blue color of the other strains. In this case the visible spectrum has a pronounced shoulder in the vicinity of 4800 Å to 5000 Å and a main peak at about 5450 Å. This species has a very significant light output at 6200 Å and about five times as much light relative to the visible in the interval beyond 6300 Å as the other four strains referred to above.

Size, dispersity and aggregation of isolated secretory (chromaffin) granules. D. B. Sattelle, D. J. Green, K. H. Langley and E. W. Westhead.

The size, dispersity and calcium-induced aggregation of adrenergic secretory vesicles (chromaffin granules) has been studied by means of laser light scattering. Chromaffin granules were isolated from bovine adrenal medulla tissue by differential centrifugation in 0.3 M sucrose (all sucrose solutions were buffered: 10 mM HEPES, pH 7.0). Granules were purified either by sedimentation through 1.6 M sucrose (sucrose granules), or by sedimentation through a 0.3 M sucrose-ficoll-D_2O mixture with a density similar to 1.6 M sucrose but isosmotic with the granules (ficoll granules). Catecholamine/protein ratios of 0.55 (sucrose granules) and 0.27 (ficoll granules) were determined.

Granules were resuspended at various concentrations in buffered sucrose and illuminated by an He-Ne laser ($\lambda_0 = 632.8$ nm). The scattered light was detected by a photomultiplier and the autocorrelation function of the intensity fluctuations $[G(\tau)]$ was obtained using a digital correlation computer. All samples were polydisperse as judged by the departure of $G(\tau)$ from a single exponential. Ficoll granules were more polydisperse than sucrose granules and showed considerable aggregation when resuspended without prior filtration. After filtration through a millipore filter (0.65 μm pore size), the Z-averaged diffusion coefficient (D_z) was calculated from $G(\tau)$. The following mean diameters were computed from $D_{z,20,w}$ (extrapolated to zero concentration): 4000 (±325) Å for sucrose granules in 0.3 M sucrose; 1700 (±225) Å for sucrose granules in 1.6 M sucrose; 3200 (±150) Å for ficoll granules in 0.3 M sucrose.

Both swelling and aggregation of granules were investigated by noting changes in D_z and the scattered intensity (I). Granules resuspended in hyposmotic solutions (0.15 M NaCl, KCl) showed a rapid decrease in D_z and a steady decline in I, indicating swelling of the granules and loss of contents. Calcium-ion induced aggregation of chromaffin granules was noted at a threshold of 2-10 mM calcium. This was detected as an increase in I, a decline in D_z and an increase in polydispersity of the sample. Laser light scattering emerges as a sensitive probe of the hydrodynamic properties of isolated secretory (chromaffin) granules.

Maturational changes in amphibian oocytes injected with oocyte fractions obtained from the surf clam. ALLEN W. SCHUETZ AND DOUGLAS SAMSON.

Effects of oocyte extracts obtained from the surf clam (*Spisula solidissima*) on meiotic maturation events in amphibian (*Rana pipiens*) oocytes were investigated. Clam oocytes were collected by centrifugation, diluted with distilled water and homogenized. Following centrifugation supernatant fractions were collected and subsequently injected, with or without further dilution, into large, defolliculate, immature (germinal vesicle stage) amphibian oocytes. Amphibian oocytes were maintained *in vitro* in amphibian Ringer's solution and monitored over a 24-hour period for maturational changes prior to and subsequent to fixation. Disintegration of the germinal vesicle was observed in some of the oocytes injected with the highest concentration of clam oocyte; however, abnormal changes in the oocyte surface and cytoplasm were also noted. Lesser concentrations of clam oocytes did not induce germinal vesicle breakdown but inhibited progesterone-induced germinal vesicle breakdown. Heating destroyed the inhibitory effects of clam oocyte extracts and appeared to enhance the effects of progesterone in inducing oocyte maturation. Acceleration of the time of nuclear breakdown and an increase in the incidence of nuclear breakdown was noted in amphibian oocytes injected with heated extracts. Divalent ionophore (A-23187) induced activation responses in progesterone-treated oocytes injected with heated but not unheated extracts of clam oocytes. These results indicate that clam oocytes contain physiologically active components which affect the meiotic process in amphibian oocytes.

Research supported by grants from NSF and NIH.

Retinal spatial interactions observed in responses of eel (and frog) ganglion cells. ROBERT SHAPLEY AND JAMES GORDON.

A diverse set of visual stimulus patterns were presented to excised eyecups from the eel *Anguilla rostrata.* Nerve impulse discharge was measured with glass micropipettes placed near ganglion cells or into optic nerve fiber bundles. Responses to periodic stimulation were averaged with a microcomputer. The nature of spatial summation in the retina was determined from the spatial dependence of the sensitivity to a contrast reversal sine grating pattern. A sinusoidal dependence of sensitivity on position of the grating should be expected if neural signals were summed linearly. This is observed only in some eel ganglion cells, denoted X cells, as in the mammalian retina. The spatial distribution of sensitivity was mapped with a thin bar modulated in luminance above and below the mean luminance. Typically, there were spatially separated regions with antagonistic responses, *e.g.*, 'on'-center cells with 'off' surrounds or *vice versa.* Consistent with this, the response to diffuse light was weaker than the response to an optimally located bar. The receptive field center ranged in size from 250 microns up to 1 mm. Some fields were not circularly symmetric. The spatial extent of excitation and inhibition was also measured by determining the spatial frequency sensitivity function with drifting sine gratings as the stimuli. Particularly striking was the fall-off in sensitivity to low spatial frequency gratings in most eel X ganglion cells, due to the clashing of center and surround signals. Using diffuse monochromatic light flashes against the standard 0.1 foot candles background, we found that some eel ganglion cells gave qualitatively different responses to different wavelengths, *i.e.,* they were color opponent cells.

This work was supported by grants from the National Eye Institute, EY–1472 and EY–188.

A new view of the postsynaptic membrane of frog muscle: scanning electron microscopy after collagenase digestion. DAVID M. SHOTTON, JOHN E. HEUSER AND THOMAS S. REESE.

Collagenase digestion of fresh neuromuscular junctions permits the removal of presynaptic nerve terminals without altering the acetylcholine sensitivity of the underlying postsynaptic membrane (McMahan, Spitzer, and Peper, 1972, *Proc. Roy. Soc. London Ser. B,* **181**: 421; Betz and Sakmann, 1973, *J. Physiol.,* **230**: 673). We have used the scanning electron microscope to study the postsynaptic membrane exposed by this treatment. Frog cutaneous pectoris muscles were digested overnight at room temperature with 1 mg/ml collagenase (Worthington CLS) in frog Ringer's containing curare, tetrodotoxin, and antibiotics. After aldehyde fixation, the partially separated muscle fibers were impregnated with osmium by alternate soaks in saturated aqueous thiocarbohydrazide and in 1% OsO_4. This permitted them to be observed after critical point drying without further metal coating. The postsynaptic folds, which lie in parallel grooves approximately 2 μm wide on the surfaces of muscle fibers, are usually freed of overlying tissues by the collagenase treatment. The junctional folds are sometimes rounded but usually have flat tops, often with a small furrow along their midline. Typically, the folds are aligned across the postsynaptic grooves at spacings of 0.3–0.5 μm, but in some places Y-branched folds are abundant while in others the folds run parallel to the axis of the groove. Highly irregular patterns are frequent, especially where several postsynaptic grooves interconnect. Ends of grooves have progressively shallower and less regular folds. Small patches of very shallow folds sometimes occur on the convex muscle membrane adjacent to normal grooves, but it is not apparent whether these were formerly covered by a nerve terminal. The pattern of postsynaptic folds is thus more variable than was indicated by thin sections. Subtle changes in the structure of these folds during synaptogenesis or induced by denervation or disease can now be investigated directly with the scanning electron microscope.

Albumin causes inhibition of synaptic transmission at the frog sartorius neuromuscular junction. L. M. SPINDEL AND A. L. POLITOFF.

Bovine serum albumin (BSA) and ovalbumin decrease the amplitude of frog sartorius end-plate potentials (EPPs) according to a concentration-dependent time course; 50 mg/ml cause a 40–50 % fall in 10–14 min. At this dose BSA did not significantly change the postsynaptic response to iontophoretically applied pulses of acetylcholine, the interval between stimulus and peak of the EPPs, the duration of the EPPs or the amplitude of the sciatic nerve action potential. A calcium electrode was used to measure the concentrations of free calcium ions. Log-log plots of EPP amplitudes against the measured free calcium ion concentration showed that albumin-treated preparations had lower EPPs at any given free calcium concentration when compared with the EPPs of preparations not exposed to albumin. This effect was greater at pH 6.5. Considering that albumin does not bind acetylcholine and does not cross the cell membrane, this result suggests that albumin may be acting on a release mechanism located on the external surface of the presynaptic membrane.

Supported by NIH grant 1 R01 NS–11588.

Voltage clamp currents from squid giant axons clamped with their action potentials. MICHAEL E. STARZAK AND RICHARD J. STARZAK.

The net current through a space-clamped squid giant axon is generally assumed to be zero during an action potential. This assumption permits the reconstruction of the action potential and its associated ionic currents from the quantitative equations for step clamp data.

To determine the effectiveness of space clamp in a system with external guard electrodes, the giant axon of the squid, *Loligo pealei*, is voltage clamped using the action potential itself as the clamping potential. The stimulated action potential of the axon is stored in digital form in the 1024 memory locations of a Tracor Northern NS570 signal averager. The digital to analog converter of the averager then reproduces this action potential in analog form for the voltage clamp. A pulse generator synchronizes the signal averager action potential output with a correction pulse which produces the proper voltage configuration for the baseline and undershoot during the duration of the clamp. An inverting attenuator is used for fine adjustment of the action potential amplitude. During the experiment, the transmembrane potential, V_{diff}, reproduces the action potential. The associated net current is observed as the central external electrode with a calibrated current to voltage converter. Finite outward currents of 0.2–0.5 mA/cm^2 are observed indicating deviations from complete space clamping which will produce zero net current. Variation of the action potential amplitude for the clamp with several axons produces no major reduction in the observed currents. Essentially all the current remains after external application of artificial sea water with 120 nM TTX indicating that this residual current arises primarily in the potassium channels. A small capacitative transient is also observed with a maximum at the maximum slope of the action potential during its rising phase. The action potential clamp technique is an extremely sensitive null probe for the effectiveness of space clamp.

This work was supported by Public Health Service Grant NS–11676.

Heterogeneity in the sea urchin (Strongylocentrotus purpuratus) *histone gene repeat detected by S_1 nuclease digestion of reassociated chromosomal DNA.* David J. States and Laurence H. Kedes.

The histone genes in sea urchin (*Strongylocentrotus purpuratus*) are tandemly repeated several hundred times. They can be digested with endonuclease Hind III into 7 kilobase units, each containing all of the five individual histone genes. The sensitivity of these repeat units to S_1 nuclease (a single strand specific nuclease) has been assayed by sizing the reaction products using gel electrophoresis. The histone sequences were located within the gel by Southern transfer and hybridization *in situ* with labeled histone DNA prepared from chimeric *E. coli* plasmids pSp2 and pSp17. The repeat units from native sperm DNA were relatively resistant to S_1 and no specific cleavage products were observed. After melting and reannealing, the repeats were more sensitive to S_1 and a specific set of products were observed. This demonstrates that there are specific regions of heterogeneity within the repeat units resulting in S_1 sensitive heteroduplex loops in reassociated DNA. The sizes of the cleaved fragments appear to map the heterogeneity into spacer regions rather than coding sequences.

This work has been supported by NSF grant no. PCM 76–09315 and the Veterans Administration.

Histone gene reiteration frequency in the surf clam Spisula solidissima. Robert E. Steele, Peter A. Merrifield and Joan V. Ruderman.

The histone genes have been shown to be highly reiterated in several species of sea urchin. These large numbers of genes facilitate synthesis of the large quantities of histones required by the rapidly cleaving embryo. Since the embryo of *Spisula* also cleaves at a very rapid rate, it was of interest to determine whether the histone genes in *Spisula* are also highly reiterated. We have isolated a fraction of RNA from polyribosomes of cleavage stage embryos which is approximately 9S in size and is the predominant RNA species being synthesized throughout cleavage. When this RNA was added to a cell-free translation system from wheat germ, the products comigrated electrophoretically with *Spisula* histones. These results strongly suggest that this RNA fraction consists of the messenger RNA's for histones. In order to obtain a measure of the reiteration frequency of the histone genes, the kinetics of hybridization of this RNA fraction in the presence of a vast excess of reassociating DNA were examined. Comparison of the rate of hybridization of the RNA to the rate of reassociation of single-copy DNA sequences indicates that the DNA sequences coding for histone mRNA's are present in no more than 100 copies per haploid genome. The actual number of copies is probably substantially less than 100. This is in contrast to the sea urchin in which the histone genes are present in 400–1200 copies per haploid genome.

This work was supported by N.S.F. grant #PCM76–09315 to the Embryology Course at MBL. R.E.S. is an N.S.F. Graduate Fellow. P.A.M. is the recipient of a predoctoral scholarship from the National Research Council of Canada. J.V.R. is a Jane Coffin Childs post-doctoral fellow.

Effect of substance P on synaptic transmission at the frog neuromuscular junction. ANTOINETTE STEINACKER.

The neuromuscular junction of the frog was chosen as a model synapse in which to study the mechanism of action of substance P since first, the neurophysiology of the synapse is well studied; secondly, the transmitter at the synapse is established as acetylcholine; thirdly, the ionic environment of the preparation can be manipulated; and fourthly, reaction kinetics can be studied at the post synaptic membrane. Standard frog Ringer's containing 0.4 mM Ca^{++} was used for control measurements and substance P (10^{-5} M) added for experimental testing. In the Ringer's containing substance P (10^{-5} M) miniature end-plate potential (MEPP) frequency was reduced by more than 80% and showed little or no recovery over a 20–40 minute time course even though control Ringer's was replaced after 10 minutes. MEPPs and evoked end-plate potentials (EPPs) both decreased in amplitude with the EPP showing the largest decrease. Quantal content (EPP/MEPP) was reduced up to 50%. The EPP and MEPP amplitude reduction returned to control values in 20–40 minutes. The effects of substance P was primarily presynaptic and could be explained by a substance P induced reduction of calcium in the presynaptic terminal. Therefore the Ca^{++} concentration in the Ringer's was increased to 3.6 mM which caused no change in control MEPP frequency or amplitude but blocked the effect of substance P on these parameters. Recent data which show that substance P (10^{-6} M) produces a 50% decrease in ^{45}Ca uptake by rat brain synaptosomes and a 100% increase in ^{45}Ca uptake by mitochondria (A. Blitz, K. Dasse, D. Sack, personal communication) would result in a decreased Ca^{++} in the presynaptic terminal and supports our findings showing that substance P has a potent inhibitory effect on synaptic transmission which is calcium dependent.

Differential protein synthesis and utilization during ciliogenesis and regeneration in sea urchin embryos. R. E. STEPHENS.

Pulse labeling with ^{14}C-leucine, hypertonic deciliation, fractionation of axonemes by differential solubilization, and autoradiographic analysis of SDS-PAGE-resolved components reveals that the onset of ciliogenesis is marked by the *de novo* synthesis of numerous "architectural" proteins of the 9 + 2 axoneme. The synthesis of these components continues, some at reduced rates, after full growth of cilia while the synthesis of tubulin nearly ceases. Deciliation results in the enhanced synthesis both of these minor components and of tubulin. The A- and B-tubulin dimers, derived from the respective subfibers, have essentially identical specific activity. Subsequent regrowth in cold leucine demonstrates substantial pools of most of the "architectural" proteins but at least two such proteins (nexin and component-20) are made quantally and in limiting amount in response to each regeneration. Such second regeneration cilia (whose pools were labeled during the first regeneration) have a decreased specific activity of B-tubulin (15–20%) and an increased specific activity of A-tubulin (25–30%), indicating a limited pool of the former but an apparent retarded synthesis, delayed activation, or initial compartmentalization of the latter. This 45% difference in the specific activity between the two tubulin dimer pools offers independent evidence that chemically unique tubulin dimers form the structurally unique subfibers. The limited, quantal synthesis of microtubule-associated proteins may be a control mechanism for ciliary elongation. Of particular interest in this regard is component-20, the major protein associated with the sarkosyl-resistant ribbons remaining after dissolution of the A-tubules. Such ribbons serve as junction points for B-subfiber and radial spoke attachment and limiting their length would consequently limit the length of the axoneme. Component-20 represents less than 2% of the total axonemal protein but during regeneration it is the major labeled species; the fact that it co-migrates with the tubulins on many gel systems would indicate caution in interpreting data solely in terms of tubulin synthesis.

Supported by NIH grants GM 20644, GM 21040, and GM 70164.

A new cystophorous cercaria from Nassarius trivittatus. HORACE W. STUNKARD.

Snails were collected at Quissett Harbor and isolated in a search for infection by larvae of digenetic trematodes. In a collection of about 150 *Nassarius trivittatus*, one specimen, taken June 20, shed hemiurid cercariae every day until it was killed on August 16 to obtain the asexual generations of the parasite. The cercariae swam vigorously, with the long tail in advance, at all levels in the water. There was no response to light. The cercariae were hardy; they lived for four days at room temperature and for ten days at 11–12° C. When released from the snail, the body was encased in a thin, flexible cyst-wall, but under pressure it emerged, leaving the tail attached to the empty cyst. There were six large rediae in the digestive gland of the snail. One was apparently exhausted and contained only ten cercariae; the others contained hundreds of cercariae. The tissues of the snail were intact and showed little injury as a result of the infection. The cercariae did not accumulate in the hemocoel but emerged from the gills soon after they had left the rediae. The cercariae were ingested by *Acartia tonsa* and developed in the hemocoel of the copepod. Stages from recently ingested cercariae to large naturally acquired metacercariae were obtained from *A. tonsa*. The large metacercariae closely resemble small juveniles of *Tubulovesicula pinguis* (Linton, 1910), a common parasite of *Menidia menidia*, and probably belong to that species. Eggs of *T. pinguis* have been embryonated and fed to *N. trivittatus* in the attempt to establish an experimental infection. This experiment is still in progress.

Investigation supported by NSF BMS–74–14534.

Computer simulation of the empirically determined diel feeding rhythm of Daphnia. JAWAHAR L. TIWARI, JAMES F. HANEY AND BRUCE J. PETERSON.

To examine the nature and magnitude of the filtering activity of *Daphnia* and its effects on primary and secondary production in lake ecosystems, a series of feeding experiments were done. Data were collected from three different lakes in Michigan and one lake in Alaska. Filtering rates were measured every hour for a 24-hour interval. Measurements were also made on body length of the organisms, diel vertical migration, and photoperiod. These results show that starting at sunset the filtering rate starts increasing and within 3–4 hours it reaches a maximum point. Then, gradually, it decreases and around midnight it reaches a minimum point. Between midnight and sunrise this cycle is repeated for the second time. Daytime filtering rate remains approximately constant.

Using this experimental data, a mathematical model of the diel filtering activity of *Daphnia* was developed. The model describes and predicts periodic and constant filtering patterns during night and day, respectively. Since the frequency of the oscillations during nighttime filtering is fixed (2 cycles), the mean and the amplitude of the oscillations are the only two input parameters required for the computer simulations. However, our experimental data indicate that the filtering rate during the night increases with body length, and a power function can be used to establish a statistical relationship between body length and the mean and the amplitude of the oscillations. Thus to simulate the model on the computer, the information about the distribution of body length at any time t and the daytime filtering rate (a constant) are needed. Our simulation results show that these organisms filter about 75–85 per cent of their food during the night. These results are consistent with the experimental data from the lakes.

This work was supported by the National Science Foundation under grant no. OPP–76–07929.

Dispersion patterns of spionid polychaetes at Barnstable Harbor, Massachusetts. ROBERT B. WHITLATCH.

A study of the spatial distribution patterns of seven species of polychaetes of the family Spionidae was undertaken from sediment samples collected from the intertidal sand and mud flats of Barnstable Harbor, Massachusetts. The core samples were brought to the laboratory and vertically dissected with the aid of a horizontally-mounted dissecting microscope to examine the nearest-neighbor relationships of the various organisms. By using this more refined approach, it may be possible to not only infer specific spatial dispersion patterns of marine invertebrates, but also obtain information on the exact parameters (both physical and biological) which may influence these patterns.

Preliminary data showed that *Streblospio benedicti, Spiophanes bombyx,* and *Scolecolepides viridis* were distributed in an aggregated or clumped fashion, *Polydora ligni* and *Scolelepis squamata* were randomly distributed, and *Prionospio heterobranchia* was over-dispersed. The actual patch size of the aggregated forms varied from species to species and did not appear to reflect the average size of individual species. Intraspecific spacing patterns were, however, greatly influenced by the quantity of the particulate food-resource available to individual species. As the amount of food increased at any one locality, the average distance between individuals of the same species decreased. It appears, therefore, that the density and spacing patterns of the spionids sampled are adjusted to match the density of available food in the sediments.

This research was supported by NSF grant DES72–01608 AO1 to Ralph G. Johnson.

Melanogenesis in melanocytes of ascidian embryos: quantity is predetermined independently of cell size and number of nuclei. J. R. Whittaker.

Larvae of *Ciona intestinalis* have only two melanocytes: the ocellus pigment cell and the otolith cell, both in the brain. Two bilateral cell lineages in the embryo give rise to these melanin-producing cells, one from each side, in the course of nine divisions from egg to final cell. If the embryos are permanently cleavage-arrested at 7 hr development (18° C) with 2 μg/ml cytochalasin B, the final two divisions of the lineage are prevented; yet virtually all of these embryos differentiate both tyrosinase enzyme and melanin pigment at the appropriate later times. The melanocytes of these cleavage-arrested embryos are four times the volume of the normally terminal cells, and have four nuclei, since cytochalasin blocks cytokinesis but not nuclear division. Two kinds of quanitative measurement were done on these cleavage-arrested embryos and normal control embryos in the same series. Tyrosinase was assayed in homogenates by the Pomerantz technique at 11 hr of development time, just before the beginning of melanin synthesis. Tyrosinase accumulates between 9 and 11 hr of development. The amount of melanin produced by embryos during the period of melanin synthesis (11 hr–21 hr) was measured by Whittaker's 2-[2-^{14}C]thiouracil incorporation method. In each case, the tyrosinase levels and the amount of melanin pigment synthesized were the same in both giant cells and normal cells. The extent of differentiation is obviously specified independently of either cell volume or multiplicity of genome. There is evidence from previous work that a cytoplasmic factor determining tyrosinase synthesis and melanocyte differentiation is localized in the ascidian egg and segregated appropriately during cleavage. This seems not to be a messenger RNA for tyrosinase. The present study suggests that this morphogen is providing quantitative as well as qualitative instructions. Possibly it regulates the extent of transcription from selected genes.

Supported by NIH grant HD–09201.

Roles of blood plasma and erythrocytes in secretion of inert gas into the teleost swimbladder. Jonathan Wittenberg, William Wittenberg and David Wittenberg.

Chemically inert gases (*e.g.*, nitrogen and argon) accompany oxygen and carbon dioxide in the gas mixture brought into the swimbladder. In deep-living fish, the partial pressures of nitrogen and argon in the swimbladder are commonly 10 and 0.1 atmosphere, respectively, ten times their partial pressures in sea water. We are attempting to find out how the inert gases are concentrated. The question arises: does the primary concentrating event bring about an increased plasma pN_2 and pAr; or are the red blood cells obligatorily involved, perhaps as suggested earlier, by linkage between oxygen release and inert gas transport?

Toadfish, *Opsanus tau,* were made severely anemic by repetitive exchange transfusions. An indwelling catheter placed in the dorsal aorta permitted withdrawal and replacement of blood without disturbing the free-swimming, unanesthetized fish. At each transfusion about half the blood volume could be removed and was replaced with an equal volume of toadfish serum. After four to six transfusions, the blood hemoglobin could be reduced to 0.02 to 0.2 millimole heme per liter blood. The normal blood hemoglobin level is about 2.3 millimolar. Such fish continue to secrete nitrogen and argon into the swimbladder at an undiminished rate, although the rate of oxygen secretion falls in proportion to the reduced level of circulating red blood cells. We conclude that the primary concentrating event in the secretion of inert gases takes place in the blood plasma and does not obligatorily involve the red blood cells.

Supported by Research Grant GB-42748 from the National Science Foundation.

Surface characteristics of zona-free mouse eggs before and after insemination. Don P. Wolf, Santo V. Nicosia and Luigi Mastroianni Jr.

Scanning electron microscopy (SEM) was used to examine changes in surface topography of follicular eggs and tubal ova before and after insemination. Ova were recovered from superovulated Swiss mice and denuded of cumulus cells by exposure to hyaluronidase (0.1%) in a modified Krebs-Ringer bicarbonate medium containing bovine serum albumin (20 mg/ml). Zonae were removed mechanically by aspiration through micropipettes or enzymatically with chymotrypsin (10 mg/ml) and processed for SEM. Ova whose zonae had been removed mechanically were inseminated with capacitated epididymal sperm and, at timed intervals thereafter, were removed, washed free of loosely bound sperm and fixed. The surfaces of those follicular eggs retaining vesicular nuclei were characterized by a uniform distribution of slender (0.3–0.4 μm width) microvilli up to 1.0 μm long. In contrast, the surfaces of follicular ova that had resumed meiosis, including both those with and without first polar bodies, were largely devoid of discernible microvilli but consisted of coarse folds (0.6–1.0 μm width) up to 0.8 μm long. The remaining surface of these eggs, usually conical in configuration, was smooth. Uninseminated tubal ova showed similar disparities in their surfaces, with a variable, but limited smooth area and a predominant fold-rich area. Such surface properties were not related to the method employed for zonae removal. In seminated eggs, recovered soon after penetration, displayed microvilli which were more extensive in ova with second polar bodies. Surfaces of both first and second polar bodies were entirely smooth. We suggest that the smooth surfaces observed in tubal ova correspond to those cortical granule-free cortexes overlying the meiotic spindle, described previously by transmission electron microscopy. Similarly, we propose that fold-rich areas correspond to that portion of the surface which contains cortical granules and are capable of incorporating sperm.

Supported by grants from Rockefeller Foundation 5–24938 and USPHS HD–07635.

Actively transcribing chromatin of sea urchin (A. punctulata) *visualized by electron microscopy.* Dulcine Zdunski, Barbara Hamkalo and Laurence Kedes.

As a first step toward visualization by electron microscopy of the primary transcripts of the histone genes in the chromasomes of sea urchin embryos, techniques were developed to disperse chromatin while maintaining its integrity and nascent transcripts.

According to the Oscar Miller technique, a cell lysate was layered over a cushion of 0.1 M sucrose with 10% formalin in a plexiglass chamber with a carbon-coated grid at its base. The lysate was sedimented through the cushion onto the grid. Thereafter, the grid was stained with phosphotungstic acid, rinsed in ethanol, and examined. The chromatin appeared dispersed after dissociated cells from morulae were lysed with 0.35% nonidet P40. Nucleosomes were visible at regular intervals. While most of the chromatin did not possess nascent RNP fibers, we did see areas of actively transcribing chromatin. We saw single transcription units with one or more attached RNP fibers. In addition, we saw as many as four transcription units, each containing several RNP fibers along single chromatin strands. RNP fibers up to 5.6 μ were visible. Also, released RNP fibers of similar lengths and configuration were found near the attached RNP fibers.

By assuming that the chromatin DNA was in B-configuration and that there are 200 basepairs of DNA per nucleosome, we were able to calculate a packing ratio of 1.28 ± 0.27 (s.d.) for the chromatin between attached transcripts of adjacent transcription units. The packing ratio for nontranscribing regions of chromatin was 2.06 ± 0.21 (s.d.). Thus, the packing ratio between transcription units was approximately 0.6 that of the chromatin in the nontranscribing regions. In addition to linear chromatin, we have consistently seen circular structures with circumferences ranging from 1.5 to 2.8 μ. Some of these circles have a beaded appearance; others had small projections around the circumference. We are now investigating the nature of these circles.

This work was supported in part by NSF grant no. PCM 76–09315 and the Veterans Administration.

Aspects of biological effects of tryptophan photoproducts. S. ZIGMAN AND T. YULO.

When tryptophan in aqueous solution is exposed to near-UV light, as in sunlight or artificial lamps, a series of photoproducts is formed. Several of these have been isolated by us which have shown differential biological effects. A 400·dalton product has great affinity for protein amino groups and is an enzyme inhibitor. A 300 dalton product inhibits the synthesis of chromatin components (DNA, histone, residual proteins) and other macromolecules (proteins) and other macromolecules (proteins, RNA) in sea urchin fertilized eggs. This photoproduct (PT 2_{II}) also delays or prevents mitosis in these eggs by interfering with the formation of the mitotic apparatus.

This summer, we found that interference with the synthesis of chromatin components and cell division due to PT 2_{II} also took place in the epithelial cells of dogfish (*Mustelus canis*) lenses *in vitro*. In the same experiments, PT 2_{I} was bound to the lens capsules and to the soluble and insoluble crystallins of the cortex, thereby altering their chemical and physical properties.

Further work to establish a basis for the action of PT 2_{II} was also done using fertilized sea urchin eggs. It was found that the action of H_2O_2 on these eggs was different than that of PT 2_{II}, and in fact from the action of whole UV-irradiated tryptophan. Little if any H_2O_2 was formed by exposing tryptophan to near-UV light. Colchicine effects on mitosis and synthesis of chromatin components was also differentiated from that of PT 2_{II}, and temperature-mediated polymerization of dogfish (*Mustelus canis*) brain tubulins, markedly inhibited by colchicine, was not influenced by the photoproducts. Other experiments showed that DNA-polymerase activity was insensitive to tryptophan photoproducts under cell-free circumstances.

Since it appears that neither DNA polymerase action nor tubulin polymerization in preparation for mitosis are influenced by the tryptophan photoproducts, other processes that would inhibit synthesis of macromolecules important in cell division must be influenced by tryptophan photoproducts. Energy supply reactions whose inhibition would interfere with cell division and macromolecule synthesis are now under investigation.

Supported by National Eye Institute; N. Y. State Health Res. Council; ONR/CNA contract (University of Rochester).

Continued from Cover Two

4. ***Literature Cited.*** The list of references should be headed LITERATURE CITED, should conform in punctuation and arrangement to the style of recent issues of The Biological Bulletin, and must be typed *double-spaced* on separate pages. Note that citations should include complete titles and inclusive pagination. Journal abbreviations should normally follow those of the U. S. A. Standards Institute (USASI), as adopted by Biological Abstracts and Chemical Abstracts, with the minor differences set out below. The most generally useful list of biological journal titles is that published each year by Biological Abstracts of those abstracted (most recent issue: November, 1972). Foreign authors, and others who are accustomed to use The World List of Scientific Periodicals, may find a booklet published by the Biological Council of the U.K. (obtainable from the Institute of Biology, 41 Queen's Gate, London, S.W.7, England, U.K. at £0.65 or $1.75) useful, since it sets out the World List abbreviations for most biological journals with notes of the USASI abbreviations where these differ. Chemical Abstracts publishes quarterly supplements of additional abbreviations. The following points of reference style for The Biological Bulletin differ from USASI (or modified World List) usage:

A. Journal abbreviations, and book titles, all underlined (for *italics*)

B. All components of abbreviations with initial capitals (not as European usage in World List *e.g. J. Cell. Comp. Physiol.* NOT *J. cell. comp. Physiol.*)

C. *All* abbreviated components must be followed by a period, whole word components *must not* (not strictly as USASI usage, *i.e. J. Cancer Res.*)

D. Space between all components (*e.g. J. Cell. Comp. Physiol.* not *J.Cell.Comp.Physiol.*)

E. We strongly recommend that more unusual words in journal titles be spelled out in full, rather than employing lengthy, peculiar "abbreviations" or new abbreviations invented by the author. For example, use *Rit Vísindafjélags Islendinga* without abbreviation. Even in more familiar languages, *Z. Vererbungslehre* is preferred to *Z. VererbLehre* (World List) or *Z. Vererbungsl.* (USASI). *Accurate and complete communication of the reference is more important than minor savings in printing costs.*

F. All single word journal titles in full (*e.g. Veliger, Ecology, Brain*).

G. The order of abbreviated components should be the same as the word order of the complete title (*i.e. Proc.* and *Trans.* placed where they appear, not transposed as in some Biological Abstracts listings).

H. Spell out *London, Tokyo, Paris, Edinburgh, Lisbon, etc.* where part of journal title.

I. Series letters *etc.* immediately before volume number.

J. A few well-known international journals in their preferred forms rather than World List or USASI usage (*e.g. Nature, Science, Evolution* NOT *Nature, Lond.; Science, N.Y.; Evolution, Lancaster, Pa.*)

K. The correct abbreviation for The Biological Bulletin is *Biol. Bull.*

5. ***Figures.*** The dimensions of the printed page, 5 by $7\frac{3}{8}$ inches, should be kept in mind in preparing figures for publication. Illustrations should be large enough so that all details will be clear after appropriate reduction. Explanatory matter should be included separately in legends as far as possible, although the axes should always be numbered and identified on the illustration itself. Figures should be prepared for reproduction as either line-cuts or halftones; no other methods will be used. Figures to be reproduced as line-cuts should be drawn in black ink on white paper, good quality tracing cloth or plastic, or blue-lined coordinate paper; those to be reproduced as halftones should be mounted on board, and both designating numbers or letters and scale-bars should be affixed directly on the figures. We recommend that halftones submitted to us be mounted prints made at about $1\frac{1}{2}$ times the linear dimensions of the final printing desired (the actual best reductions are achieved from copy in the range from $1\frac{1}{4}$ to 2 times the linear dimensions). As regards line-blocks, originals can be designed for even greater reductions but are best in the range $1\frac{1}{2}$ to 3 times. *All* figures should be numbered in consecutive order, with no distinction between text and plate-figures. The author's name should appear on the reverse side of all figures, and the inked originals for line-blocks must be submitted for block-making.

6. ***Mailing.*** Manuscripts should be packed flat. All illustrations larger than $8\frac{1}{2}$ by 11 inches must be accompanied by photographic reproductions or tracings that may be folded to page size.

Reprints. Reprints may be obtained at cost; approximate prices will be furnished by the Managing Editor upon request.

CONTENTS

ACHE, B. W., Z. M. FUZESSERY, AND W. E. S. CARR
Antennular chemosensitivity in the spiny lobster, *Panulirus argus:* comparative tests of high and low molecular weight stimulants.... 273

CHEVONE, BORIS I. AND A. GLENN RICHARDS
Ultrastructure of the atypic muscles associated with terminalial inversion in male *Aedes aegypti* (L.)........................... 283

FISHER, THOMAS R.
Oxygen uptake of the solitary tunicate *Styela plicata*............. 297

HARZA, T. AND LENKE MÁTYÁS
Seasonal variations of sodium and potassium concentrations in different parts of the frog myocardium.......................... 306

HERNANDORENA, A.
Effects of temperature on the nutritional requirements of *Artemia salina* (L.).. 314

HUNTER, F. R.
Permeability of trout erythrocytes to nonelectrolytes............. 322

JØRGENSEN, C. BARKER
Comparative studies on the function of gills in suspension feeding bivalves, with special reference to effects of serotonin............ 331

LEVERSEE, GORDON J.
Flow and feeding in fan-shaped colonies of the gorgonian coral, *Leptogorgia*... 344

NIMITZ, SR. M. AQUINAS
Histochemical changes in gonadal nutrient reserves correlated with nutrition in the sea stars, *Pisaster ochraceus* and *Patiria miniata*.. 357

SCHULTZ, T. WAYNE AND JOHN R. KENNEDY
Cytotoxic effects of the herbicide 3-amino-1, 2, 4-triazole on *Daphnia pulex* (Crustacea : Cladocera)................................ 370

THOMAS, J. D., M. POWLES, AND R. LODGE
The chemical ecology of *Biomphalaria glabrata:* the effects of ammonia on the growth rate of juvenile snails...................... 386

ABSTRACTS OF PAPERS PRESENTED AT THE MARINE BIOLOGICAL LABORATORY.. 398

Volume 151 Number 3

Marine Biological Laboratory
LIBRARY

THE
BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

Editorial Board

JOHN B. BUCK, National Institutes of Health
JOHN O. CORLISS, University of Maryland
DONALD P. COSTELLO, Woods Hole, Massachusetts
JOHN D. COSTLOW, Duke University
PHILIP B. DUNHAM, Syracuse University
CATHERINE HENLEY, National Institutes of Health
GEORGE O. MACKIE, University of Victoria
HOWARD A. SCHNEIDERMAN, University of California, Irvine
RALPH I. SMITH, University of California, Berkeley
GROVER C. STEPHENS, University of California, Irvine
CARROLL M. WILLIAMS, Harvard University
EDWARD O. WILSON, Harvard University

W. D. RUSSELL-HUNTER, Syracuse University
Managing Editor

DECEMBER, 1976

Printed and Issued by
LANCASTER PRESS, Inc.
PRINCE & LEMON STS.
LANCASTER, PA.

THE BIOLOGICAL BULLETIN

THE BIOLOGICAL BULLETIN is issued six times a year at the Lancaster Press, Inc., Prince and Lemon Streets, Lancaster, Pennsylvania.

Subscriptions and similar matter should be addressed to THE BIOLOGICAL BULLETIN, Marine Biological Laboratory, Woods Hole, Massachusetts. Agent for Great Britain: Wheldon and Wesley, Limited, 2, 3 and 4 Arthur Street, New Oxford Street, London, W. C. 2. Single numbers, $8.00. Subscription per volume (three issues), $22.00, (this is $44.00 per year for six issues).

Communications relative to manuscripts should be sent to Dr. W. D. Russell-Hunter, Marine Biological Laboratory, Woods Hole, Massachusetts 02543 between June 1 and September 1, and to Dr. W. D. Russell-Hunter, P.O. Box 103, University Station, Syracuse, New York 13210, during the remainder of the year.

Copyright © 1976, by the Marine Biological Laboratory

Second-class postage paid at Lancaster, Pa.

INSTRUCTIONS TO AUTHORS

THE BIOLOGICAL BULLETIN accepts original research reports of intermediate length on a variety of subjects of biological interest. In general, these papers are either of particular interest to workers at the Marine Biological Laboratory, or of outstanding general significance to a large number of biologists throughout the world. Normally, review papers (except those written at the specific invitation of the Editorial Board), very short papers (less than five printed pages), preliminary notes, and papers which describe only a new technique or method without presenting substantial quantities of data resulting from the use of the new method cannot be accepted for publication. A paper will usually appear within four months of the date of its acceptance.

The Editorial Board requests that manuscripts conform to the requirements set below; those manuscripts which do not conform will be returned to authors for correction before review by the Board.

1. ***Manuscripts.*** Manuscripts must be typed in double spacing (*including* figure legends, foot-notes, bibliography, etc.) on one side of 16- or 20-lb. bond paper, 8½ by 11 inches. They should be carefully proof-read before being submitted and all typographical errors corrected legibly in black ink. Pages should be numbered. A left-hand margin of at least 1½ inches should be allowed.

2. ***Tables, Foot-Notes, Figure Legends, etc.*** Tables should be typed on separate sheets and placed in correct sequence in the text. Because of the high cost of setting such material in type, authors are earnestly requested to limit tabular material as much as possible. Similarly, foot-notes to tables should be avoided wherever possible. If they are essential, they should be indicated by asterisks, daggers, etc., rather than by numbers. Foot-notes are not normally permitted in the body of the text. Such material should be incorporated into the text where appropriate. Explanations of figures should be typed double-spaced and placed on separate sheets at the end of the paper.

3. ***A condensed title*** or running head of no more than 35 letters and spaces should be included.

Continued on Cover Three

NOTICE TO SUBSCRIBERS

Effective with the first issue of Volume 152 (February, 1977), the subscription price for The Biological Bulletin will be raised. Subscription per volume (three issues) will be $22.00 (this is $44.00 per year for six issues); single numbers will be $8.00 each. Continuing 1977 subscriptions for which payment has actually been received before November 1, 1976 will be honored at the old rate.

Vol. 151, No. 3 December 1976

THE

BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

A CYTOCHEMICAL FINE STRUCTURE STUDY OF PHAGOTROPHY IN A PLANKTONIC FORAMINIFER, *HASTIGERINA PELAGICA* (d'ORBIGNY)

Reference: *Biol. Bull.* **151**: 437–449. (December, 1976)

O. ROGER ANDERSON AND ALLAN W. H. BÉ

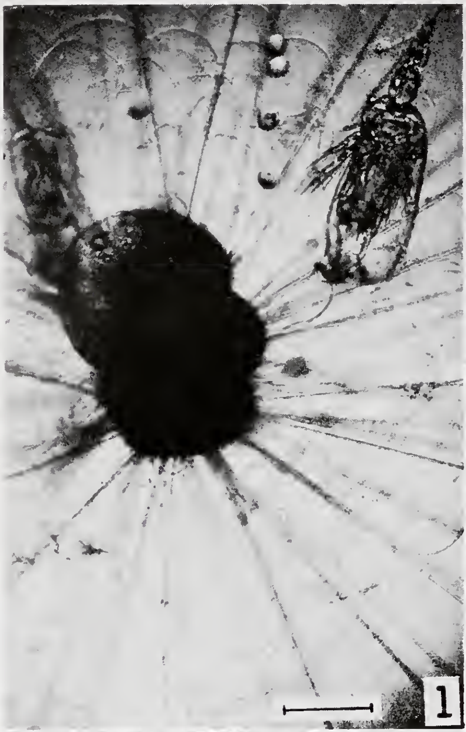
Lamont-Doherty Geological Observatory of Columbia University, Palisades, New York 10964

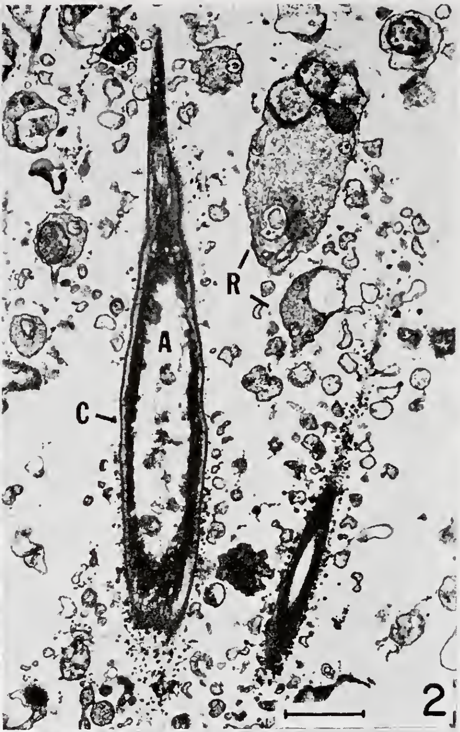
In spite of the significant role of planktonic foraminifera in marine food webs, little is known about their nutrition. Part of the lack of knowledge can be attributed to the difficulty in capturing uninjured specimens, which hampered previous attempts to culture them in the laboratory. By contrast, benthic foraminifera have been cultured for some time and their nutrition has been examined more thoroughly (*e.g.* Lengsfeld, 1969; Lee, 1974).

Recently, planktonic foraminifera have been successfully maintained in the laboratory for sufficiently long periods of time to enable observations of gametogenesis (Bé and Anderson, 1976) and nutritional studies. Although it is commonly assumed that planktonic foraminifera are predominantly herbivorous, it has been found that most of the spinose species are carnivorous and that only a few species are omnivorous. Omnivorous species sometimes capture diatoms and other small algae, but also prey on small crustacea which are snared within their weblike rhizopodia.

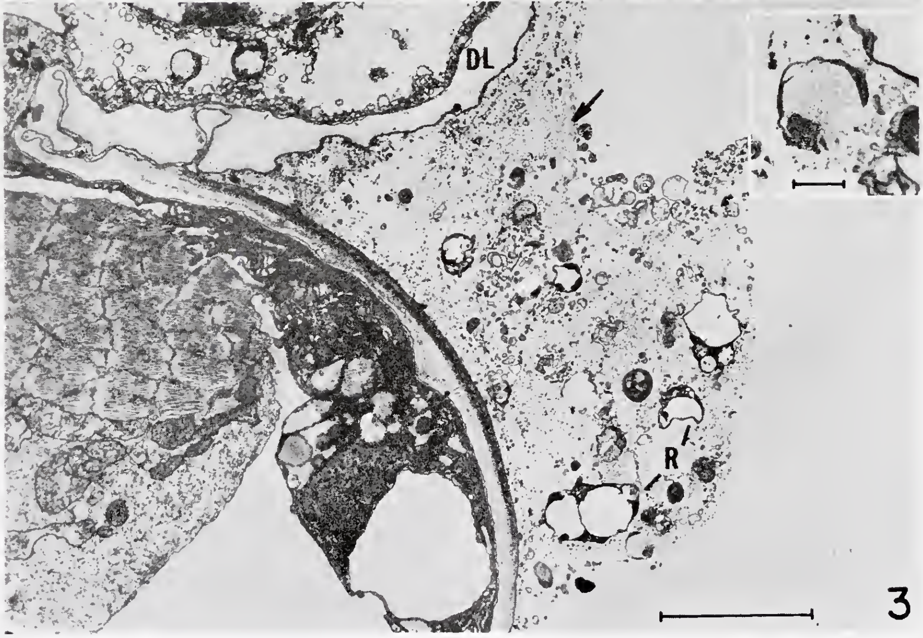
Rhumbler (1911) published colored drawings of planktonic foraminifera [*Hastigerina pelagica, H. digitata, Globigerina triloba* (= *Globigerinoides sacculifer*)] containing fusiform, pink, striated particles in their endoplasmic vacuoles. He identified these striated bodies as copepod muscle fibers. However, little attention seems to have been given to this pioneering observation. The present research has confirmed that these particles are pieces of muscle from prey.

Hastigerina pelagica was selected for the present study due to its abundance at the collection site, its robustness, and its ready acceptance of *Artemia* nauplii as food organisms in laboratory cultures. The nutritional habits of laboratory-cultured organisms and of specimens immediately obtained from the ocean are reported. The mode of capturing prey and the activity of rhizopodia in invading and engulfing prey tissue is examined. The cytochemistry of lysosomal activity in phagocytosis and digestion is presented.


1



2



3

MATERIALS AND METHODS

Collection and maintenance of specimens

Hastigerina pelagica was collected in glass jars by SCUBA diving near the surface approximately ten miles southeast of St. David's Battery, Bermuda, on July 9, 1975. Single specimens were maintained at the Bermuda Biological Station in pint-size jars containing millipore-filtered sea water. They were kept at 25° C under fluorescent illumination of a 12L:12D cycle. Three specimens of *H. pelagica,* which were fed *Artemia* (brine shrimp) nauplii cultured in the laboratory, were examined. One was fed eight hours and again two hours before fixation on July 28 to determine the early and late stages of prey capture and digestion. The remaining two were fed eight hours before fixation and were used for cytochemical studies of digestive enzyme secretion and distribution.

Preparation for electron microscopy

Specimens were fixed for 30 minutes at 3° C in 3% glutaraldehyde buffered with 0.2 M cacodylate buffer (pH 8.0). The cytochemical method of Gomori (1952) was used to detect lysosomal acid phosphatase. A NaF control was used to confirm the validity of the enzyme reaction product. Specimens prepared for transmission electron microscopy and cytochemistry were suspended in an agar matrix after glutaraldehyde fixation (and, where appropriate, after cytochemical preparation) to preserve the delicate rhizopodial strands. Specimens were post-fixed in 2% osmium tetroxide in 0.2 M cacodylate buffer (pH 8.0), stained with 2% uranyl acetate in 10% aqueous ethanol (except the cytochemical specimens), dehydrated in an ethanol series, cleared in propylene oxide and embedded in Epon. Ultra-thin sections were obtained with a Porter-Blum MT-2 ultramicrotome and collected on copper grids. Some of the sections were post-stained with Reynold's lead citrate. Sections were observed with a Philips EM-200 microscope operated at 60 kV.

RESULTS

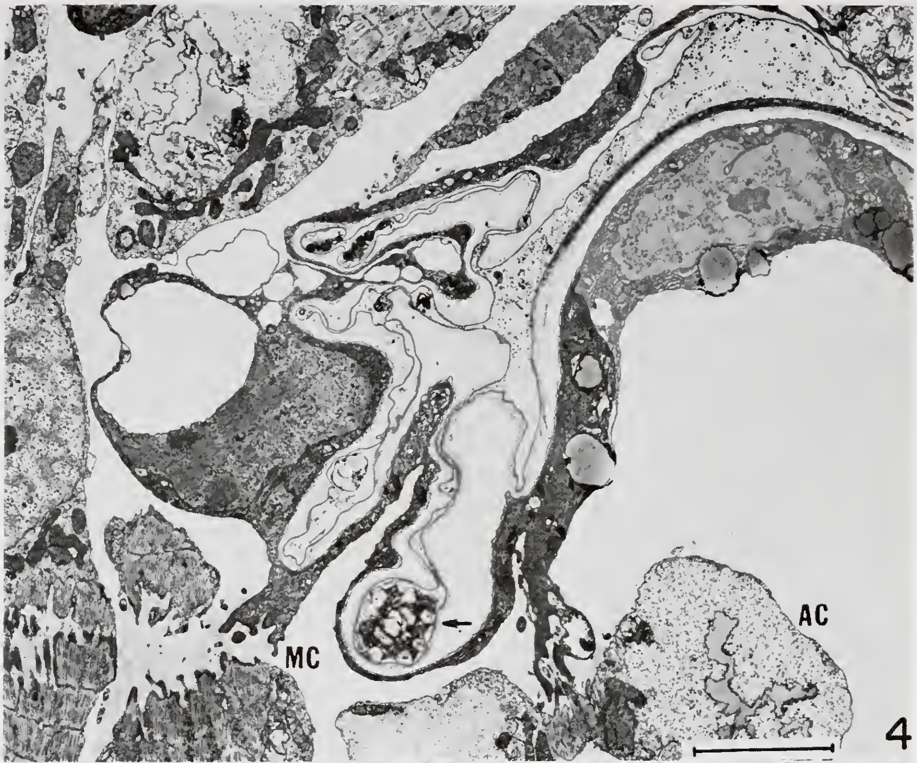
Light microscopic observations

Hastigerina pelagica is unique among the planktonic foraminifera in its possession of a bubble capsule, resembling a mass of soap bubbles, that completely surrounds its shell (Fig. 1). An adult *H. pelagica* has a shell length of about

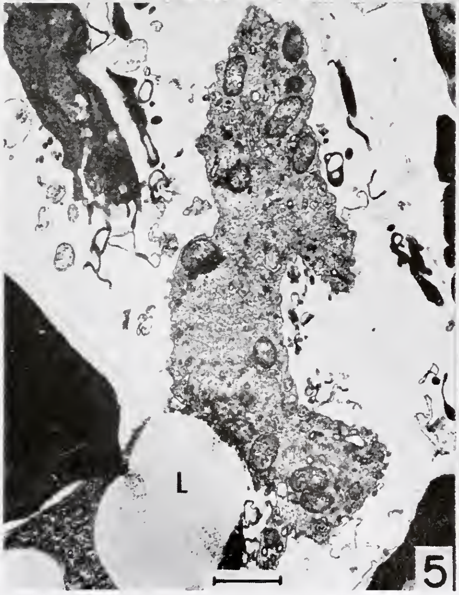
FIGURE 1. A snared copepod has been drawn into the bubble capsule of *Hastigerina pelagica* shortly after being collected. Closely packed bubbles surround the shell (lower left) and its radially arranged spines. Scale bar equals 0.25 mm.

FIGURE 2. An electron micrograph of a section through the appendages (A) of a snared *Artemia* nauplius shows the abundance of rhizopodia (R) near the surface (c) of the prey; scale bar equals 1 μm.

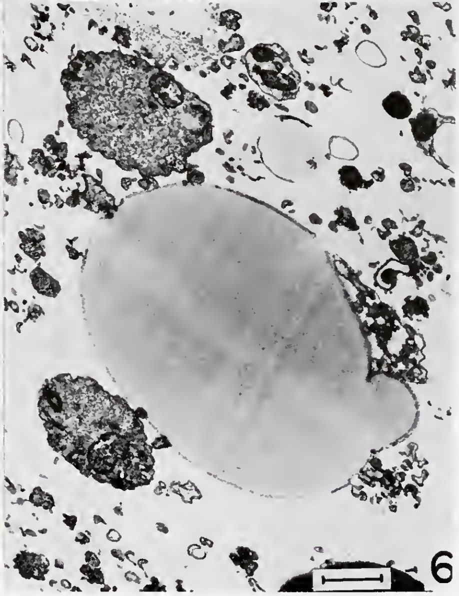
FIGURE 3. The surface of a captured *Artemia* nauplius is covered with adhesive substance (arrow) secreted on the double-layered cuticle (DL) by rhizopodia (R). Insert at upper right shows the release of adhesive substance from a vacuole within a rhizopodium. Scale bar equals 4 μm; inset scale bar equals 0.5 μm.



4



5



6

1 mm and a spine length of about 12 mm. Hence, the total diameter of a floating specimen is about 25 mm. The capsule in adult specimens has an average diameter between 2 and 3 mm and consists of numerous bubble compartments that are stacked on top of each other. In general, the bubbles are spherical and approximately 200 μm in diameter, but they may be flattened along contact surfaces between bubbles.

A large proportion of captured *H. pelagica* specimens have one or more copepods lodged inside their bubble capsules (Fig. 1). Frequently the copepods are completely digested, as only empty carapaces remain visible. *H. pelagica's* carnivorous habit is readily demonstrated by the introduction with a pipet of copepods (*e.g., Oncaea* sp. and *Farranula* sp.) to floating specimens in culture. Healthy, freshly-collected *H. pelagica* will capture copepods when the latter come into contact with its network of rhizopodia, which extend through the capsule and occur along, as well as between, the spines.

H. pelagica and other planktonic foraminifera also feed on *Artemia* (brine shrimp) nauplii in the laboratory. When an *Artemia* touches a spine, it immediately adheres to the sticky rhizopodia and is frequently rendered helpless within seconds. Few specimens escape once they have been snared. Within a short duration, usually about 30 minutes, the crustacean is transported by the rhizopodia along the spine to the bubble capsule. As the prey is carried into the capsule, the bubbles are displaced. The crustacean can be held anywhere in the bubble capsule and is not oriented in any particular way relative to the shell aperture. The crustacean is mechanically disrupted by the rhizopodia which invade the soft tissue and begin digestion outside of the test. Some of the dislodged tissue is carried into the test where further digestion occurs.

Copepod carapaces are ejected from the bubble capsule several hours after capture and following complete digestion. This is not the case for *Artemia,* whose nauplii possess a very thin cuticle (Hootman, Harris and Conte, 1972); portions of the cuticle are ejected continuously as invasion and digestion of prey tissue occurs.

Electron microscopic observations

Rhizopodial attachment to prey outside the bubble capsule. The first stage of capture involves rhizopodial attachment to the prey (Figs. 2–4). Fine strands of rhizopodia (R in Figs. 2 and 3) are congregated in the vicinity of the small appendages (A) of the prey. Many of these strands are so fine that they cannot be resolved with the light microscope, but are clearly visible with the electron microscope. The cuticle (C) of the *Artemia* nauplius appendage exhibits a thin electron dense outer layer (*ca.* 29 μm thick) and a thicker irregular layer beneath it about 0.2 μm thick. Considerable variation occurs in morphology and diameter

---

FIGURE 4. Rhizopodia (arrow) congregate within the inner-most recesses of the *Artemia* cuticle and eventually penetrate into the underlying adipose tissue (AC) and muscle tissue (MC). Scale bar equals 5 μm.

FIGURE 5. A rhizopodium containing characteristic tubular mitochondria attaches to an extruded lipid droplet (L). Scale bar equals 1 μm.

FIGURE 6. An extruded lipid body is surrounded by rhizopodia which have transported it away from a lysed cell; scale bar equals 1 μm.

of the rhizopodia. The larger strands, approximately 102 μm diameter, contain a granular cytoplasm with small tubular mitochondria (*ca.* 0.5 μm diameter). Occasional microtubules, and endoplasmic reticulum are also observed. Mitochondria in the cytoplasm within the shell are about 1 μm in diameter and therefore are somewhat larger than those seen in small rhizopodia. Very fine rhizopodia (*ca.* 0.05–0.2 μm) contain no discernible organelles. The small rhizopodia occur abundantly near the surface of the prey. These are probably very small branches from the larger rhizopodia.

Rhizopodia in the vicinity of broad surfaces on the *Artemia's* body secrete a fibrous mass of adhesive substance which adheres to the prey (Fig. 3). This material undoubtedly strengthens the attachment of the rhizopodia to the surface of the prey and reinforces the rhizopodial strands by providing a matrix between them. Evidence for secretion of the adhesive substance by rhizopodia is presented in the enlarged inset in Figure 3. A vacuole containing a fine fibrillar mass of adhesive substance has ruptured releasing its content. Rhizopodia of various diameters occur within the adhesive matrix and some of them contain mitochondria and large empty vacuoles.

The cuticle surrounding the body region in *Artemia* nauplii consists of a double layer (DL) of thin electron dense lamellae with a fairly electron translucent space between them. The surface of the cuticle is rugose and forms deep crevices and fissures. Immediately beneath the exoskeleton is a layer of epithelial tissue connected to muscle cells or adipose tissue. Very fine rhizopodia (arrow in Fig. 4) and the adhesive substance penetrate deep into the crevices of the cuticle (Figs. 3 and 4). Muscle fiber cells (MC) and adipose cells (AC) containing lipid are also visible within the body cavity. The remarkable tenacity with which the foraminifera holds its prey can be attributed to the massiveness of the rhizopodial attachment, their deep penetration into crevices of the prey and their reinforcement through secretion of the adhesive substance.

Rhizopodial invasion of prey tissue. The extension of rhizopodia into *Artemia's* cuticle crevices eventually leads to penetration within the body cavity. Within eight hours after capture of prey, electron microscopic examination shows penetration of rhizopodia inside the cuticle and surrounding epithelial cells containing lipid reserves (in Figs. 5 and 6). The rhizopodia are readily distinguished from prey tissue by their irregular margin and tubular mitochondria which are typically protozoan. Many of the cells within the body cavity where rhizopodia have invaded appear moribund, because they possess an electron dense granular cytoplasm, contain few intact organelles and some of their lipid droplets appear to be extruded through the lysed cell membrane.

Ingestion of tissue fragments by rhizopodia. Rhizopodial invasion of the prey is followed by engulfment of prey tissue within food vacuoles. Lipid droplets released from *Artemia* cells within the body of the prey are surrounded by rhizopodia (Fig. 6). Some produce prong-like projections that invade the surface of the lipid droplet. Adhesive substance is also released within the body cavity of the prey. The lipid droplets, masses of *Artemia* cells and shattered segments of cuticle are transported by the rhizopodia away from the body of the prey. The digestible substances such as lipid and cell material are sequestered within rhizopodial vacuoles and carried within the aperture of the foraminifer. Large sheets of cuticle (arrows in Fig. 7) dislodged from the prey are shown being transported away by the rhizopodia and are apparently discarded at the periphery of the ectoplasm. Food vacuoles containing cellular material from the prey are carried into the earliest chambers of the foraminiferal shell, indicating that ingestion and digestion occur throughout the intrashell cytoplasm. A small chamber (Fig. 8) which is one of the oldest and farthest removed from the aperture contains a large food vacuole (FV) approximately 20 μm diameter and smaller ones nearby that are 4.7 μm diameter. The shell (S) has been decalcified during preparation for electron microscopy, but the organic lamellae within the wall remain.

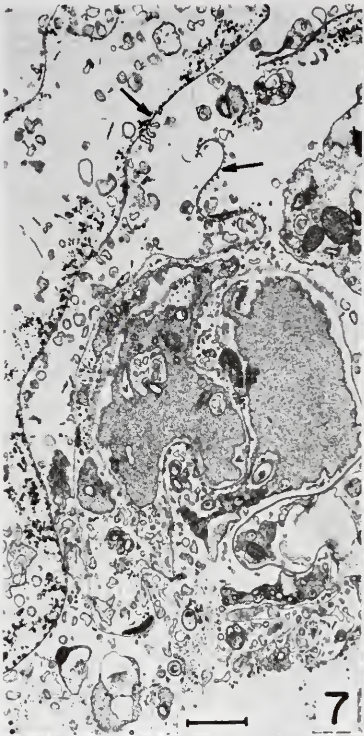
Formation of digestive vacuoles. The food vacuoles within the endoplasm are converted to digestive vacuoles as indicated by lysis of sequestered food particles and the presence of lysosomal enzymes marked by acid phosphatase reaction product (X) (Fig. 9). The larger digestive vacuoles are 4.5–6.0 μm in diameter, which agrees with the diameter of food vacuoles observed in Figure 8. The small vacuoles containing reaction product are primary lysosomes and have a typical diameter of 0.5–0.7 μm.

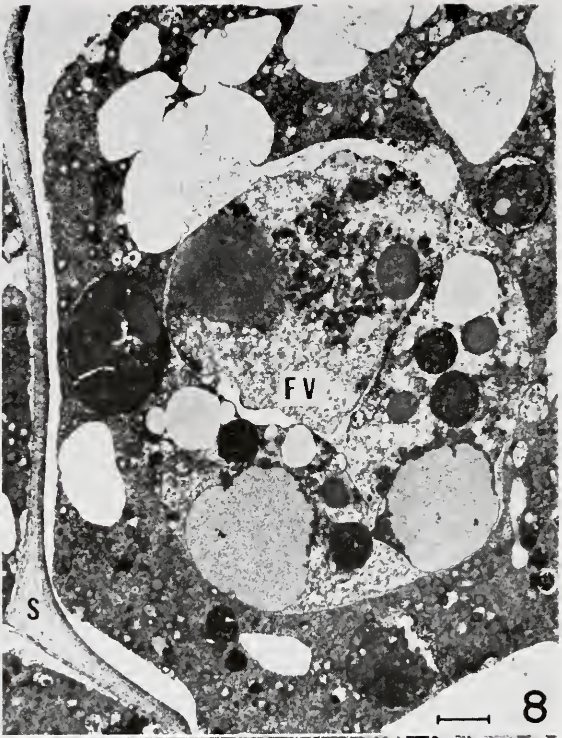
In addition to digestive vacuoles contained in the endoplasm, conversion of food vacuoles into digestive vacuoles while they are still in the rhizopodial network can sometimes be seen.

Origin and role of adhesive substance. The adhesive substance used to capture prey originates in the Golgi apparatus within the endoplasm. In conventional, stained electron microscopic preparations of *H. pelagica,* the Golgi contains fine fibrous deposits in the cisternae and distended saccules on its maturing face (the surface containing enlarged vacuolar spaces which appears as the concave surface in Fig. 10). The fibrous deposits are also present in vacuoles (V) near the Golgi in Figure 10. These adhesive-containing vacuoles are apparently transported outward into the rhizopodia to aid in capture of prey. The Golgi, therefore, serves remarkably diverse secretory roles. It secretes lysosomal enzymes as part of the digestive apparatus and can also produce adhesive substance (possibly mucoid).

Lysosomal distribution. Lysosomes (Ly) secreted in the endoplasm within the shell are carried into the rhizopodia outside of the shell (Fig. 11) and in the thin cytoplasmic partitions forming the bubble capsule. The large number of lysosomes in the rhizopodia and the presence of occasional masses of cytochemical reaction product (X) in the lacunae between the rhizopodial strands (Fig. 12) suggest that extracellular enzymes may be secreted to kill and help dislodge cells from the prey. Masses of prey tissue (CM) are present in the lacunae and are already in contact with lysosomal enzymes. It is not possible to determine how the enzymes (marked by reaction product) were released into the lacunae. They may have been secreted there directly by primary lysosomes or they could be excess enzymes released during defecation of residual digestive vacuoles.

Association with microamoebae. During the course of the cytochemical investigation, a small amoeboid cell was noticed (*ca.* 5 μm in diameter) among the foraminiferal rhizopodia (A in Fig. 11). This is clearly not a part of the rhizopodial network, since it possesses its own nucleus and presents a typical amoeboid cytoplasmic fine structure. One of its large vacuoles contains reaction product (X) and amorphous material that appears to be in a late stage of digestion. Additional reaction product appears in small vesicles on the opposite side of the cell and these look like primary lysosomes. This may be a free-living amoeba that





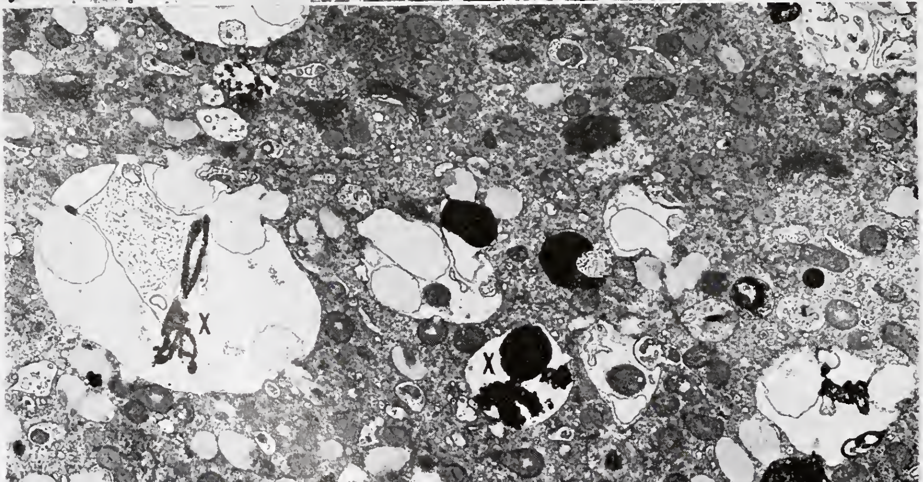


FIGURE 7. Rhizopodia surround sheets of cuticle (arrows) that have been torn from the prey and are being carried away to be eventually discarded. Scale bar equals 1 μm.

has established a commensal association with the foraminifer. There is no evidence that these small amoebae are parasites. Moreover, samples have been taken of foraminiferal rhizopodial cytoplasm with surrounding culture fluid and inoculated into sterile F/8 medium (Guillard and Ryther, 1962). The inoculum produced an algal bloom and numerous small amoebae which appeared to feed on the algae. It is concluded that the amoebae in the rhizopodial network are capable of an independent existence but have assumed a scavenger role in the foraminiferal ectoplasm, engulfing small particles of food not taken by the foraminifer. The presence of these microamoebae within the ectoplasm of *H. pelagica* may indicate an interesting ecological adaptation between two environmentally compatible Sarcodina.

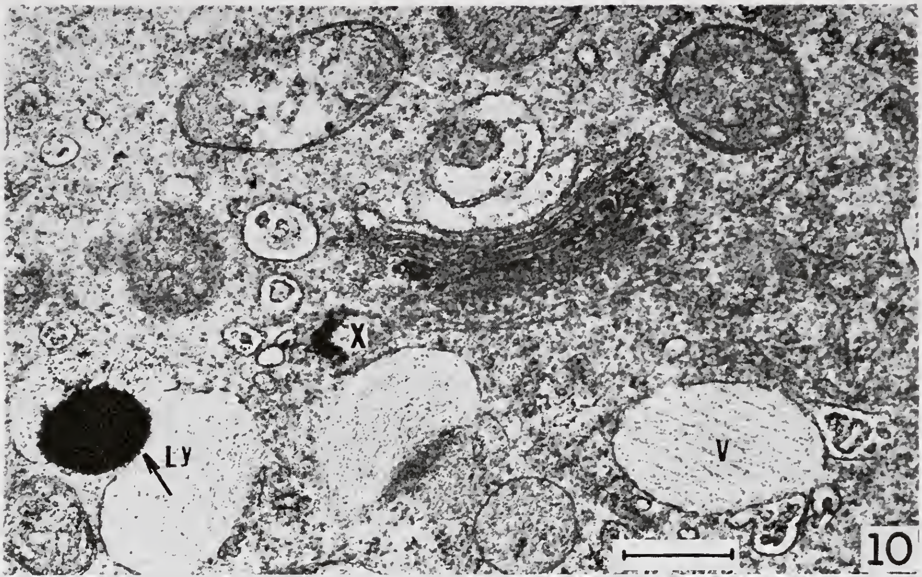
Discussion

The ingestion of food particles by enclosing them in cytoplasmic vacuoles (phagocytosis) is a well-established nutritional mode among the Sarcodina (Jepps, 1956; Hall, 1965; Grell, 1973). However, little is known about the mechanisms of food capture and ingestion in floating species that produce rhizopodial networks. The rhizopodia-bearing species are clearly different from lobopodia-bearing species such as amoebae, which surround their prey or pinch it into small fragments before ingestion. *H. pelagica* illustrates the facile mechanism of rhizopodia-bearing species in snaring prey, dislodging manageable segments of tissue and engulfing food particles in food vacuoles in the rhizopodial network. This network extends far beyond the perimeter of the organism's shell and forms a three-dimensional, sticky web that efficiently tangles prey coming within its bounds. The rapid cessation of struggle by the prey suggests that the foraminifer secretes a narcotizing agent, but there is no direct evidence of it at present.

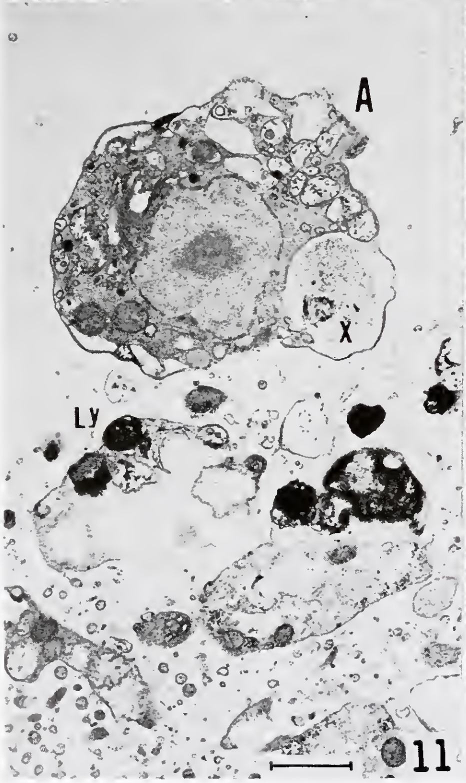
The presence of an adhesive substance may serve several roles other than reinforcement of attachment. The adhesive material occurs in Golgi secreted vacuoles and sometimes is observed in close proximity to lysosomes. The fine fibrous secretion emitted in the prey tissue may help to contain extra-cellular enzymes at the site of attack and thus increase their efficiency and conserve their concentration. Moreover, it is known that many digestive enzymes are acid hydrolases which have a pH optimum near 5. Sea water is alkaline and is not a suitable environment for acid hydrolase activity unless some mechanism is established to create microenvironments with acid pH. If the adhesive material contains acid mucopolysaccharides, they may create acidic microenvironments surrounding the fibrous substance that enhances digestive enzyme activity. Extracellular acid phosphatase reaction product has been observed in regions of rhizopodial attack on prey and in lacunae among the rhizopodia outside of the shell.

FIGURE 8. A food vacuole (FV) occurs in the innermost portion of cytoplasm within a small chamber of the foraminiferal shell (S). Scale bar equals 2.5 μm.

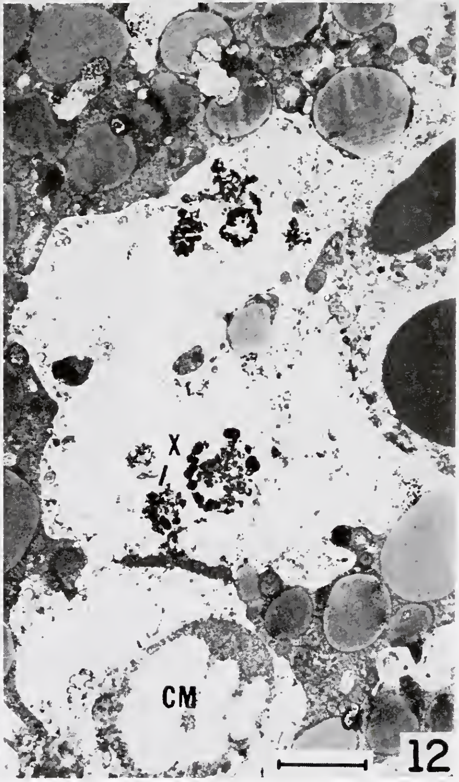
FIGURE 9. Digestive vacuoles containing digestive enzymes (indicated by cytochemical reaction product, X) contain remains of digested prey. The digestive vacuoles are formed from food vacuoles by fusion with lysosomes containing the digestive enzymes; scale bar equals 1 μm.



10



11



12

FIGURE 10. Lysosomes originate in the Golgi apparatus as shown by the presence of cytochemical reaction product (X) within the peripheral saccules of the Golgi and in nearby

Some digestion of the prey can occur in the lacunae in addition to digestion within digestive vacuoles in the endoplasm. Foraminifera purge their cytoplasm of undigested waste material by carrying it out of the aperture in residual vacuoles which stream along the rhizopodia and are released at some distance from the shell (Anderson and Bé, 1976). It is possible that defecation near the aperture will also contribute residual digestive enzymes to be used in preliminary digestion of newly ingested food within rhizopodial lacunae. If such reuse of digestive enzymes does occur, it demonstrates a remarkable cellular economy.

Lengsfeld (1969) has suggested that digestion in the benthic foraminifera *Allogromia laticollaris* occurs solely in the branching rhizopodial lacunae rather than in digestive vacuoles. However, her observations are based on noncytochemical preparations, and therefore it is difficult to assess the validity of her hypothesis. In *H. pelagica,* there is evidence that the digestive vacuoles are completely membrane-bound, since sequential sections taken through a digestive vacuole region show little evidence of canal-like connections among the vacuoles. Moreover, cytochemical evidence for digestion in these vacuoles is presented in this study.

It is not possible to determine what proportion of the digestive vacuole activity is due to hydrolases secreted by the foraminifer as opposed to endogenous lysosomal enzymes of prey cells released during cell death. Thus, part of the digestion may be due to autolysis and part to predator hydrolases secreted into the digestive vacuoles. The presence of Golgi-secreted lysosomes in phagotrophic protozoa has been well established by electron microscopic cytochemical studies (Goldfischer, Carasso, and Favard, 1963; Elliot and Clemmons, 1966; Stoltze, Lui, Anderson and Roels, 1969).

There is a remarkably selective activity of the rhizopodia during capture and engulfment of prey. Some rhizopodia sever large masses of cuticle from the prey which are torn away and carried some distance. However, little of this nondigestible material is transported into the intrashell cytoplasm, as occurs with the digestible soft tissue. When small prey (several μm in size) containing a shell are captured, they may be carried whole into the foraminiferal cytoplasm where they appear within a digestive vacuole. The basis for this selective behavior by the rhizopodia is not known but certainly constitutes one of the most remarkable and potentially illuminating adaptations exhibited by these unicellular organisms. There is no fine structure characteristic that separates food-carrying rhizopodia from those dislodging sheets of cuticle. It must be presumed that the differential response is determined by chemotactile stimulation. The nature of membrane chemoreceptors, if present, has not been investigated to the best of our knowledge. The intensity of rhizopodial activity in feeding also appears to be modulated according to the nutritional state of the foraminifer. When it is well-nourished, invasion of prey tissue and its ingestion may last for many hours. The

---

secretory vesicles (Ly). Adhesive substance also occurs abundantly in vacuoles (V) in the Golgi region; scale bar equals 0.5 μm.

FIGURE 11. A microamoeba (A) containing a digestive vacuole (X) was observed living amidst the foraminiferal rhizopodia containing lysosomes (Ly); scale bar equals 1 μm.

FIGURE 12. Digestive enzymes marked by reaction product (X) are released into lacunae with the rhizopodial network surrounding masses of cellular material (CM) dislodged from the prey; scale bar equals 2 μm.

specimens used in this study were well nourished and some digestion of prey was observed as much as eight hours after snaring it. In poorly nourished specimens, invasion and digestion of prey can occur within a few hours after it is snared.

The web-like shroud of rhizopodia in *H. pelagica* and their remarkably facile ability to snare prey and separate food particles from nondigestible substances represents an elegant adaptation to a floating marine existence. The wide range of food accepted and the ability of the foraminifers to snare and subdue motile prey of nearly half their size bear witness to their robustness and adaptability to diverse nutritional demands.

We express appreciation to John Hacunda and Saijai Tuntivate-Choy, who assisted with laboratory cultures and light microscopy preparations. This research was performed in part at the Bermuda Biological Research Station, St. George's West 1–15, Bermuda, and was supported by National Science Foundation Grants DES 75–14374, DES 75–19026 and DES 76–02202. Additional support was provided by a S. L. Wright Fellowship from Bermuda Biological Station. The Philips-Norelco Company generously donated a microscope to the Bermuda Biological Station during the course of this research. This is Lamont-Doherty Geological Observatory Contribution no. 2404 and Bermuda Biological Station Contribution no. 635.

Summary

The fate of *Artemia* (brine shrimp) nauplii offered as food to *Hastigerina pelagica* in laboratory cultures was determined using light and electron microscopy.

Contact between prey and foraminiferal rhizopodia leads to immediate attachment. Adhesive substance is secreted and rhizopodia invade crevices of the prey, penetrate beneath the cuticle, and begin disruption of prey tissue. Some tissue masses and cells are dislodged and digestion is begun outside of the test as indicated by lysosomal enzymes surrounding partially degraded prey tissue within spaces created by surrounding rhizopodia. Dislodged prey tissue is sequestered into food vacuoles and carried into the intrashell cytoplasm where digestion also occurs. The digestive enzymes are secreted by the Golgi apparatus in membrane-bound vesicles (lysosomes) which are carried throughout the cytoplasm and fuse with the food vacuoles to produce digestion. The carapace or cuticle of digested prey is discarded and undigested waste material in residual vacuoles is defecated at the periphery of the rhizopodial network.

LITERATURE CITED

Anderson, O. R. and A. W. H. Bé, 1976. The ultrastructure of a planktonic foraminifer, *Globigerinoides sacculifer* (Brady), and its symbiotic dinoflagellates. *J. Foraminiferal Res.,* **6**: 1–21.

Bé, A. W. H. and O. R. Anderson, 1976. Gametogenesis in planktonic foraminifera. *Science,* **192**: 890–892.

Elliott, A. M. and G. L. Clemmons, 1966. An ultrastructural study of ingestion and digestion in *Tetrahymena pyriformis*. *J. Protozool.,* **13**: 311–323.

GOLDFISCHER, S., N. CARASSO AND P. FAVARD, 1963. The demonstration of acid phosphatase activity by electron microscopy in ergastoplasm of the ciliate *Campanella umbellaria. J. Microsc.,* **2**: 621-628.

GOMORI, G., 1952. *Microscopic histochemistry: principles and practice.* University of Chicago Press, Chicago, 189 pp.

GRELL, K. W., 1973. *Protozoology.* Springer-Verlag, Heidelberg, 554 pp.

GUILLARD, R. R. L. AND J. H. RYTHER, 1962. Studies of marine planktonic diatoms: I. *Cyclotella nana* Hustedt, and *Detonula confervacea* (Cleve) Gran. *Can. J. Microbiol.,* **8**: 229–239.

HALL, R. P., 1965. *Protozoan nutrition.* Blaisdell, New York, 87 pp.

HOOTMAN, S. R., P. J. HARRIS AND F. P. CONTE, 1972. Surface specialization of the larval salt gland in *Artemia salina* nauplii. *J. Comp. Physiol.,* **79**: 97–104.

LEE, J. J., 1974. Towards understanding the Niche of Foraminifera. Pages 207–260 in R. H. Hedley and C. G. Adams, Eds., *Foraminifera,* Vol. 1. Academic Press, New York.

LENGSFELD, A. M., 1969. Nahrungsaufnahme und Verdauung bei der Foraminifera *Allogromia laticollaris. Helgolaender Wiss. Meeresunters.,* **10**: 385–400.

JEPPS, M. W., 1956. *The Protozoa, Sarcodina.* Oliver and Boyd, Edinburgh, London, 183 pp.

RHUMBLER, L., 1911. Die Foraminiferen (Thalamophoren) der Plankton Expedition; Teil 1—Die Allgemeinen Organisations Verhältnisse der Foraminiferen. *Plankton-Exped. Humboldt-Stiftung, Ergebn.,* **3(L, c)**: 1:331.

STOLTZE, H. J., N. S. T. LUI, O. R. ANDERSON AND O. A. ROELS, 1969. The influence of the mode of nutrition on the digestive system of *Ochromonas malhamensis. J. Cell. Biol.,* **43**: 396–409.

Reference: *Biol. Bull.* **151**: 450–466. (December, 1976)

OCULAR DEVELOPMENT AND INVOLUTION IN THE EUROPEAN CAVE SALAMANDER, *PROTEUS ANGUINUS* LAURENTI

JACQUES P. DURAND

Laboratoire souterrain du C.N.R.S., 09410 Moulis, France

Proteus anguinus Laurenti is a well-known urodele amphibian, and it is the only real cave-dwelling vertebrate in Europe. Its biology remains, however, a relatively unknown field of study. In spite of the interest it has aroused in naturalists, there remain many contradictions and inexactitudes in studies of which this urodele has been the object. These contradictions are found in many basic questions concerning the biology of the animal, its method of reproduction, its immature or larval state, and its adaption to underground life.

On this last point, the genesis of the rudimentary state of the eyes (see Fig. 1) is of special interest. After two centuries of research, only a very general idea has been formed of the eye of *Proteus*. It is known that it is sensitive to light. The fact remains, however, that the precise forms of ocular development are unknown, and the problem of its involution remains unsolved. Two working hypotheses emerge from the contradictory accounts of previous research: the first is that the degeneration of the eye is a secondary consequence of the adaptation to an underground environment, with a link between the disappearance of the eye and the darkness in the caves; and the second is that the ocular atrophy is a kind of arrested development, linked to a larval neotenic state.

Scarcity of material explains this situation. It is not yet known where *Proteus* reproduces naturally. Ecologically, it is known that *Proteus* comes to feed in the underground rivers of the Dinaric Alps Karst. The water-bearing Karst, where its development takes place, remains inaccessible. This present work was only possible after cultures of *Proteus* had been established in the laboratory.

Materials and Methods

A breeding culture of *Proteus* was begun at the underground laboratory of the C.N.R.S. in 1957 by Professor Vandel and his assistants. From observations on this breeding culture, it is known that oviparity is regular and that the development and growth of each animal continues until it reproduces in its turn around the fourteenth year (Vandel and Durand, 1970). There is no true metamorphosis, and each specimen of *Proteus* can be considered to be a mature animal (Fig. 2a). It has also been possible with the breeding cultures to study and experiment upon hundreds of specimens of embryos, larva, and young.

There is no need to set out the now classic histological or electron microscope methods, such as the silver impregnation of Bodian, nor to describe in detail methods of biometry, preparation of graphic reconstruction, or of the translucencies to examine skeletal parts, all of which can be found in many works.

The experiments consisting of exposure to daylight and to artificial light were carried out in aquariums where the water was kept under the same conditions as the water where the control animals were found. The lighting was at levels of intensities between 100 and 6000 lux. The range of artificial lighting was between 800 and 1200 nm.

In all that is relative to the cultures, transplants, regeneration baths and hormone injections, the classic methods of experimental embryology were employed for the organotypic cultures and the xenoplastic transplants; the embryos used were at the tail-bud stage. From many species, two (*Pleurodeles waltlii* Michaelles and *Euproctus asper* Dugès) gave particularly satisfactory results and were retained as hosts or as donors. The animals were sacrificed from a period of a few days to a year and a half after each experiment.

RESULTS

DEVELOPMENT OF SENSE ORGANS

Organogenesis of the eye

As is the case with all vertebrates, one can distinguish the appearance of the presumptive eye field of *Proteus* at the time when the ectoblast is differentiated into epiblast and neuroblast. At the neurula stage (stage: 30–45 days, 6–7 mm at 11.6° C; Durand, 1971) the presumptive visual field comprises a part of the neural crests and a portion of the floor of the medullary groove. At the end of neurulation (45–50 days, 7–8 mm), the head is formed and the optic vesicles evaginate from the proencephalic region of the neural tube.

At the tail bud stage (50–55 days, 8–9 mm; Vandel, Durand and Bouillon, 1964), the optic vesicle is greatly enlarged and is joined to the ectoderm.

Between 60–70 days of development (10–11 mm), the lenticular placode is differentiated from the basilar epidermis, and the primary optic vesicle is transformed into a secondary optic cup. One notices the incipient dissociation between the single layer of cells of the future pigmented layer and the thickening of the retinal layers. At the anterior limb bud stage (70–80 days, 11–13 mm) where the lens is still attached to the basal epidermis, the retinal layer is deeply refolded (Fig. 2b). At the following stage (80–90 days, 13–15 mm) the lens which has separated from the ectoderm appears in the form of a closed epithelial vesicle in which the cellular nuclei are situated at the periphery.

At the stage of the cylindrical limb bud (90–100 days, 15–17 mm), the organization of the eye and its attendant envelopes is developed. One can see the organization of the retina and the optic nerve is differentiated. The corneal ectoderm is reduced to two layers of cells. At the time of the formation of the fingers of the anterior limb (stage 100–110 days, 17–18 mm), the inner plexiform layer of the retina appears. The lens differentiates at a slower rate than the retina when compared to other salamanders (*Pleurodeles, Euproctus, Ambystoma*). This is somewhat anomalous morphogenetically.

Between 110 and 120 days (19–24 mm), slightly before hatching, the eye pigment appears as a black semi-circle. The cerebral plexiform layer of the neural retina is spread out and divided into multipolar and bipolar cells. The visual cells appear and the lumen of the lens is rejected towards the surface, while the cells of the posterior pole begin to form a nucleus. At this stage, the embryo

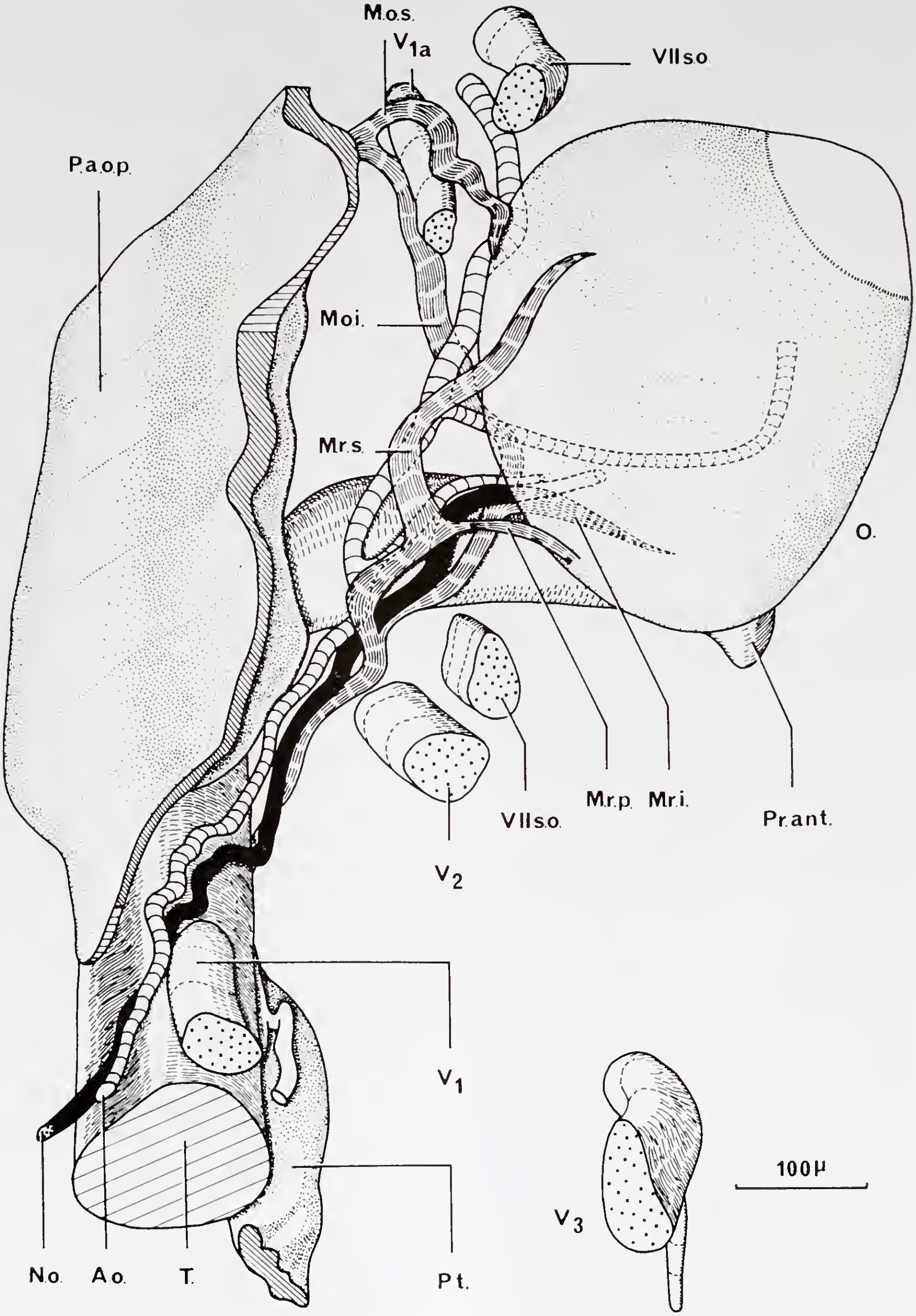

M.o.s.
V1a
VIIso.
P.a.o.p.
Moi.
Mr.s.
O.
Mr.p.
Mr.i.
Pr.ant.
VIIso.
V2
V1
V3
100µ
N.o.
A.o.
T.
Pt.

FIGURE 1. Larval orbit in *Proteus anguinus* prepared from sections by graphic reconstruction. P.a.o.p. represents the *processus orbito-parietale;* M.o.s., the *musculus obliquus superior;*

is transformed into a larva (hatching). From the exterior the gross appearance of the eye is circular and black. At its center, the clear spot of the lens is transparent. The eye is near to the state of its maximum differentiation (Fig. 2c). The external plexiform layer separates and the cells of the conjunctiva extend from the sclera in a primitive sclero-corneal limbus. The cornea remains essentially in a "dermoid cornea" state.

On the course of larval life (24–40 mm, 16–120 days after hatching), the eye grows but does not undergo further differentiation. A characteristic regressive development is the thickening of the supraoptic ectoderm (Fig. 2d) and the appearance of lytic vacuoles in the lens tissue (Fig. 3b and 3c).

At four months post-emergence, the larval stage ends. The individual enters the juvenile phase of development, but the eye will always retain its generally embryonic appearance which continues in the adult animal (Fig. 2e).

Analysis of ocular organogenesis

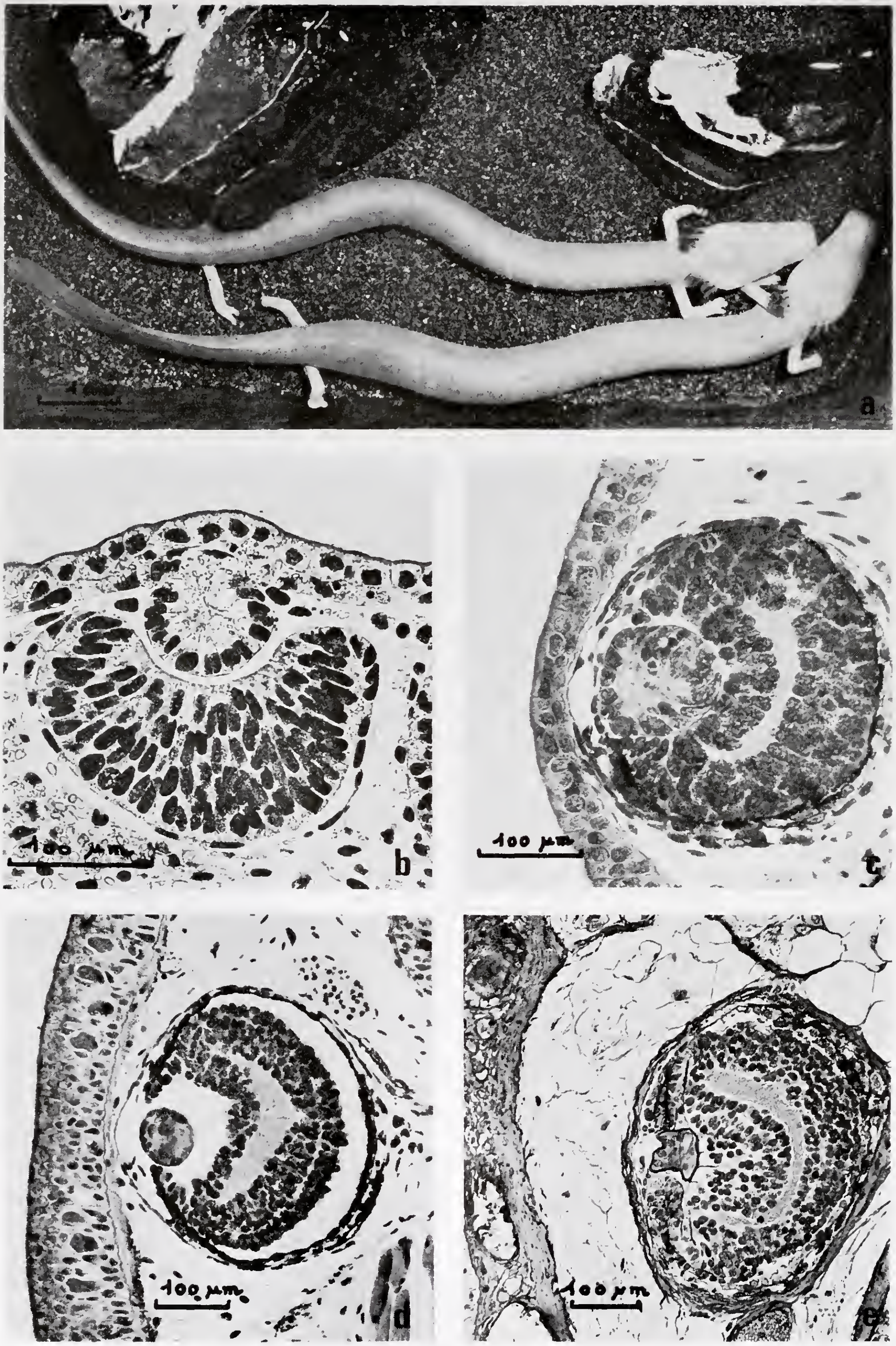
The modes of ocular organogenesis have proved to follow the normal sequence of embryogenesis which runs: archencephalic induction; evagination of the optic vesicle from the primitive diencephalon; transformation into the secondary optic cup; formation of a lens from the superficial ectoderm; and finally, differentiation of a thin and transparent dermal cornea. Such development would appear commonplace, if it were not for certain specific modifications, including the slowing up of some growth and anatomical migration and involution.

Slackening of growth. From the stage when the external plexiform layer and the expansion of the visual cells appear, the retina retains its single embryonic appearance (Fig. 2c) : its elements are fewer in number and larger in size, the lens remains enclosed within the retina, the vitreous body hardly develops and the continuous fold between the retina and pigmented epithelium persists without giving rise to an iris.

Contrary to certain hypotheses, development does not stop. The retina continues to grow: the number of cells increase from 1500–2000 to more than 10,000 over a ten-year period; the pigmented epithelium retains its ability to regenerate a lens throughout the life of the animal; the cartilaginous plates of the sclera appear late (at about 3 or 4 years) ; and finally comparison of the first stage of organogenesis between the eye of *Proteus* and the eye of *Necturus,* another Proteidae, show that the development of the optic field of *Proteus* is very slow.

Anatomical migration. The eye progressively sinks into the cephalic tissues, and then connective tissue becomes interposed between the eye and the hypodermis. The eye is subject also to a retrocaudal displacement (Fig. 1). Starting anterior to the anteorbital process of the trabecula, it later comes to occupy a posterior position in the adult.

V_1a, the *ramus ophthalmicus profondus* of the Vth cranial nerve; VIIs.o., the *ramus ophthalmicus superficiale* of the VIIth cranial nerve; M.o.i., the *musculus obliquus inferior;* M.r.s., the *musculus rectus superior;* O., the eye; Pr.ant., the *processus antorbitalis;* M.r.i., the *musculus rectus inferior;* M.r.p., the *musculus rectus posterior;* V_2, the *ramus maxillaris* of the Vth cranial nerve; V_1, the *ramus ophthalmicus* of the Vth cranial nerve; Pt., the *ossa pterygoidea;* V_3, the *ramus mandibularis* of the Vth cranial nerve; T., the *trabecula cranii;* A.o., the *arteria ophthalmica;* and N.o., the *nervus opticus.*


1 cm
a
100 µm
b
100 µm
c
100 µm
d
100 µm
e

Involution. Principally, the involution of the dermal cornea is expressed by the supraocular ectoderm becoming thick and opaque (Fig. 2d and 2e); the involution of the lens is by enantiometric allometric growth which can lead to its disappearance (Fig. 4). Thus, the study of ocular development shows the growing dissociation which exists between the fate of the derivatives of the neuroblast and those derived from the epiblast of the eye.

The initial development would appear morphogenetically normal, if the slowing of growth was not manifested (starting from the closure of the neural ridges), and if some involutive phenomena did not appear at the time of the formation of the lens. These processes are reinforced during larval development, directing the eye towards its specific modifications which vary from the classic development of the vertebrate eye.

Correlation with other sense organs

After having described the ocular development of *Proteus,* it is useful to compare its characteristics with those of the other sense organs. The organization of the olfactory apparatus is simple, and it is well developed in the adult. Only the chondrification of the olfactory capsule shows a delay comparable to that for the chondrification of the cartilaginous plates of the scleral skeleton. In contrast to the development of the eye, the development of the olfactory sac itself is normal.

The comparison between the development of the acoustic apparatus of *Proteus,* and the development of the ear of the other amphibians shows that the organogenesis of the ear is somewhat delayed (for example, it requires 1800 hours at 12° C, against 275 hours at 10° C for *Rana sylvatica*). The primary developmental stages of the eye follows the sequence of ocular development, but the later development of the ear is clearly more rapid because the internal ear is well differentiated and the otic capsule chondrified at the end of the larval stage.

It is known that the lateral line organs register vibrations of the liquid environment in which the animal is immersed. The neuromasts of *Proteus* are of a superficial type and the lateral neurosensory system corresponds to the basic type for aquatic vertebrates.

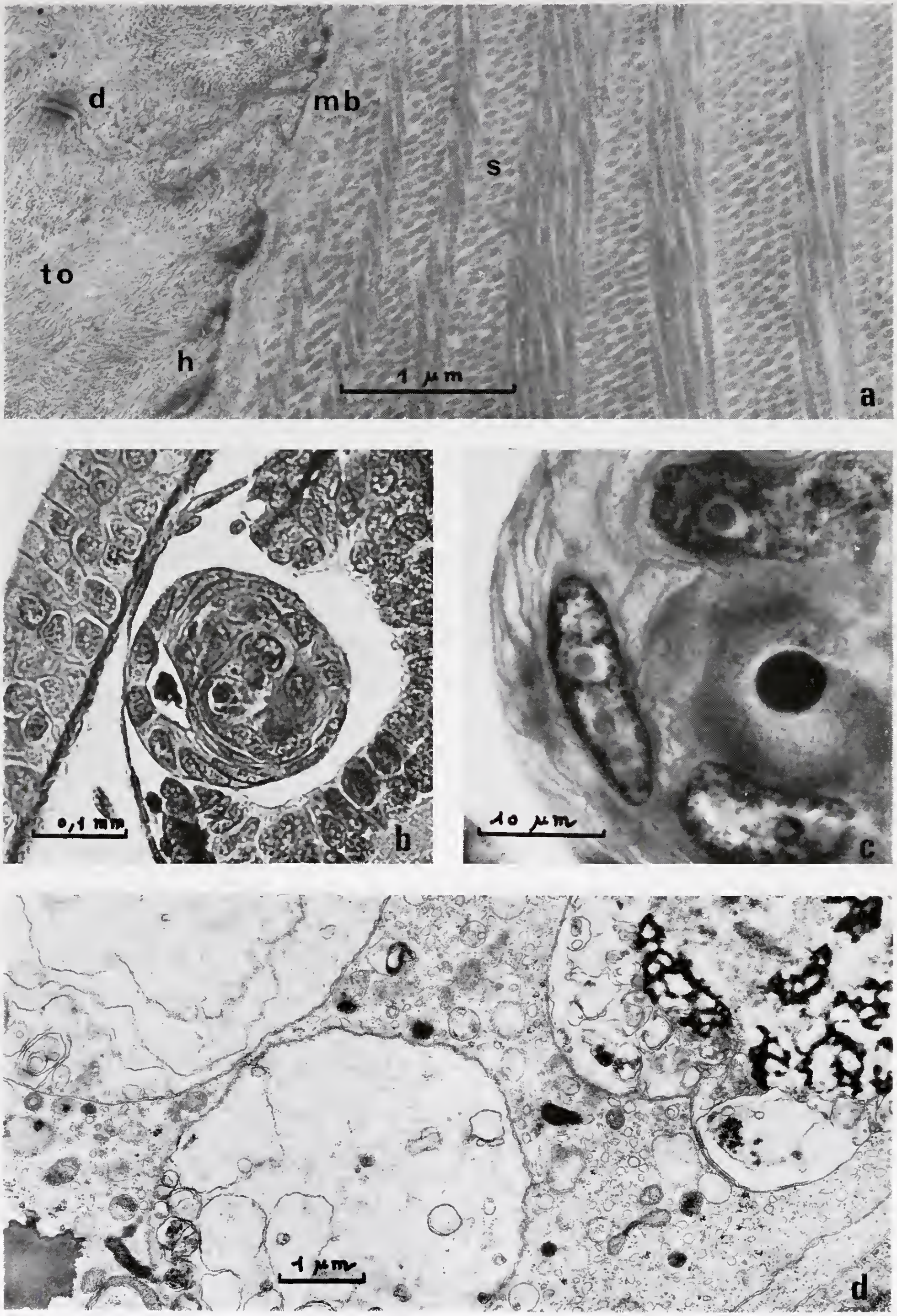
In summary, the development of sense organs of *Proteus* is slow and simple, but remains perfectly normal. One can say that the initial development of the eye follows a normal course, but that its development is extremely slow and the organ cannot attain the normal structure of the vertebrate eye. Development begins with a marked ontogenetical slowing, and finally is clearly regressive.

DEVELOPMENT OF LARVAL, JUVENILE AND ADULT EYE

Cephalic organization

Skeleton. The cranium of *Proteus* is more elongated and less massive than those of the other urodeles. One can see the stability of the chondrocranium,

FIGURE 2. a: Adult *Proteus;* b, a microphotograph of the eye at the anterior limb bud stage; c, the eye at the hatching stage; d, the eye at the larval stage; and e, the eye at the adult stage.


d
mb
s
to
h
1 µm
a
0,1 mm
b
10 µm
c
1 µm
d

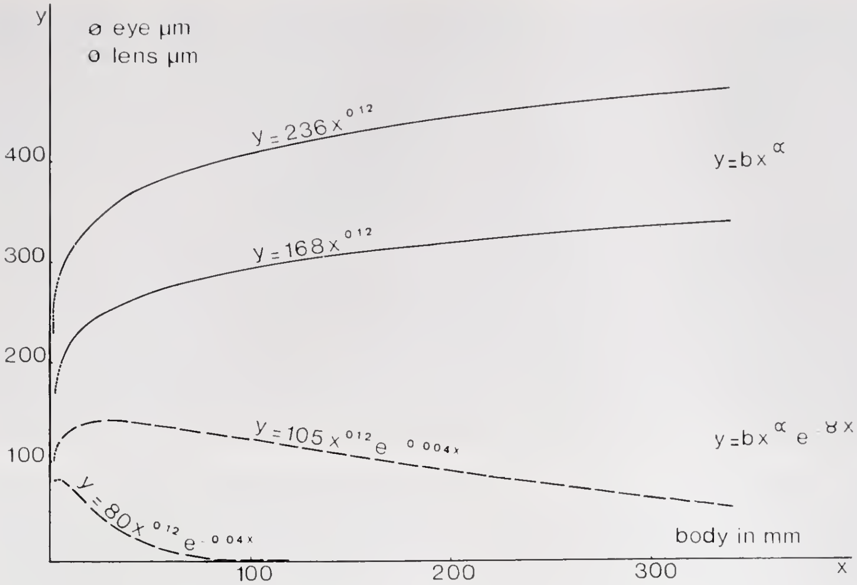


FIGURE 4. Maximal and minimal values for growth of the eye and of the lens in *Proteus* in relation to the body size. Units of the ordinate are microns, and of the abscissa body length in millimeters. Solid lines and the accompanying equations are those for the relative growth of the eye; broken lines, those for the lens.

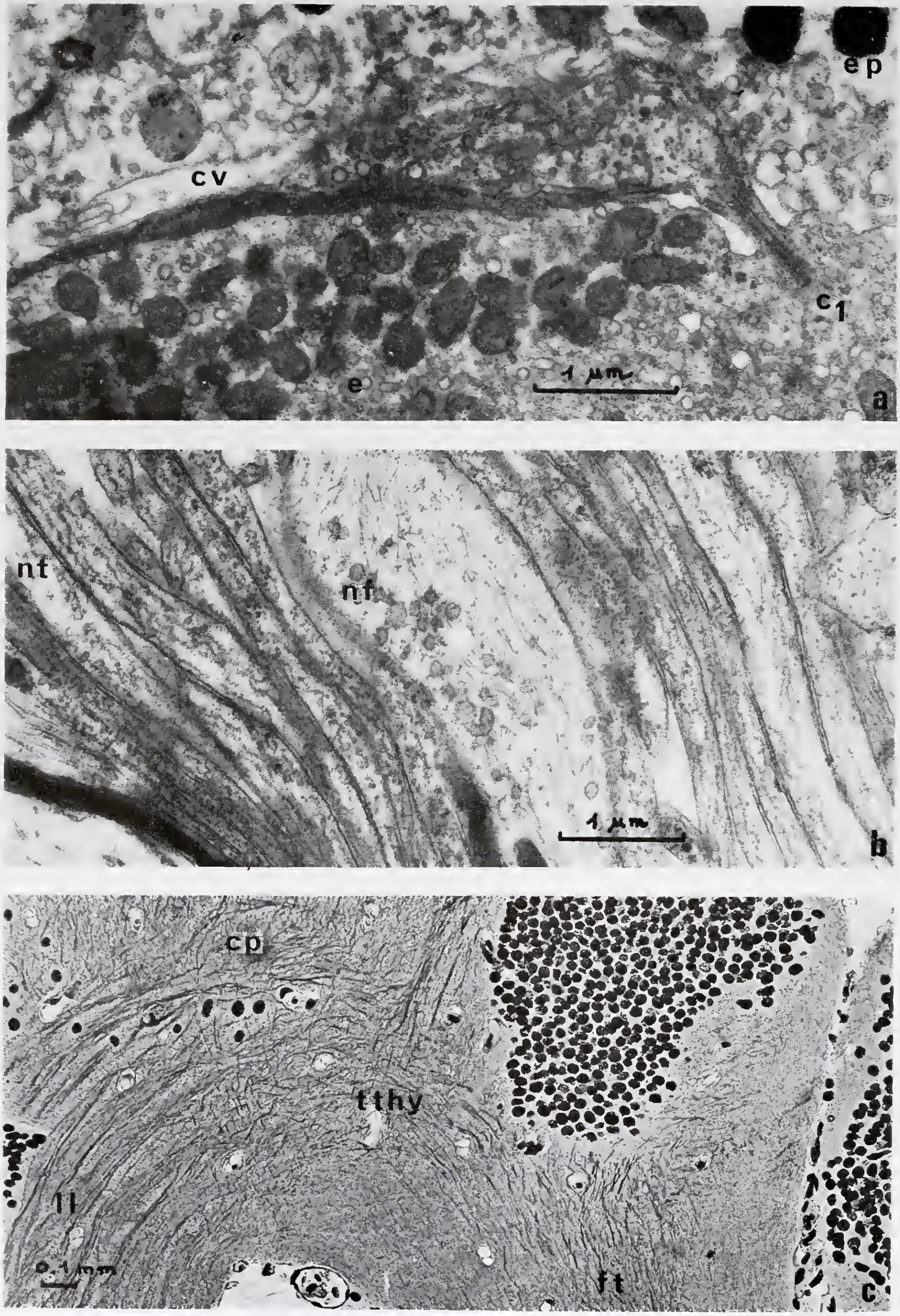
except in the nasal and orbital region where the olfactory capsules show only slight development. The scleral cartilage is frequently absent, and the orbitosphénoïd is usually absent. In general, the anterior part of the orbital cartilage is ossified and constitutes a large part of the walls of the cranium. Because of the missing skeletal pieces in *Proteus,* one observes an extension of the parietal bone to the trabecula. Such evidence from study of the skeleton, in particular the hypobranchial skull being only partially ossified, along with the low sensitivity of tissues to thyroidal hormones, does not support the view of other authors (Hawes, 1946) in hypothesizing a neotenic state, but indicates that the skeletal organization of *Proteus* is fundamentally simple.

Musculature. The muscles of the orbit are numerous, and can be grouped: the cephalic muscles into elevators and depressors of the jaw, and the muscles of the buccal floor, deep muscles and dorso-longitudinal muscles (Durand, 1971).

Nervous system. The brain of *Proteus* corresponds to the classic brain of amphibians such as *Necturus.* It presents, however, some differences: a greater elongation; its position in the posterior zones of the cranium, by the development of the olfacto-gustative regions; and the smaller development of diencephalic and mesencephalic regions. It is important to note that, contrary to certain opinions, the auricular cerebellum exists, and the acoustico-lateral area is predominant in

---

FIGURE 3a. Dermoid cornea by electron microscopy: d, dendrite; mb, basal membrane; s, stroma; h, hemidesmosome; to, tonofibril. 3b shows larval lens; c, electron micrograph of the degenerative lens; d, electron micrograph of the lens lytic vacuoles.


ep
cv
c1
e
1 µm
a
nt
nf
1 µm
b
cp
tthy
ll
0,1 mm
ft
c

the medullary centers. Lastly, the branches of the cranial nerves, with the exception of those of the trochlear (IV) and abducens (VI) can be followed.

In general, the observed simplicity concerning the cephalic organization as well as the organization of the other apparatus inclines one to think that *Proteus* is not a neotenic form, as is often said, but rather a perennibranch. This can only confirm the interpretation of Vandel (1966) who considers this animal as a relict form surviving underground.

Orbital attachment

Variations of the extrinsic musculature of the eye lie within the corresponding limits of the embryonic stages which precede the complete differentiation of these muscles. One can show a clear parallel between the state of development of the ocular muscles (reduced or absent, Fig. 1), and the weak development or the disappearance of the ocular motor nerves, and the reduction of vascularization. The elongation of the ocular muscles, of the nerves of the orbit, of the optic nerves and of the ophthalmic vessels is tied to the great elongation of the cranial structures and the relatively minor development of the diencephalic nervous system. In this connection, they indicate that throughout all of the juvenile life of the animal, said to extend through a dozen years, marked disharmonies of growth can be observed between the diverse parts of the body of *Proteus.*

Ocular tissues

Dermoid cornea. The cornea of *Proteus* is made up of two elements: a part of the original epithelium and part of the original sclera. In the electron micrograph (Fig. 3a) of the cells of the corneal epithelium, one notices the hemidesmosomes of the dermo-epithelial type connecting Bowman's membrane and the stroma to regularly arranged collagenous fibers. The scleral part of the cornea is comprised principally of fibroblasts. They delimit an anterior chamber of the eye which encloses a very finely granular material, the vitreous humor. The supraocular tegument of the larva of *Proteus* is differentiated in a transparent dermoid-cornea, comparable to that of some cyclostomes, Dipnoi and *Ichtyophis.*

Lens. The differentiation proceeds as in the case of the other vertebrates, but does not pass the stage of the embryonic lens at the time when the fibers and the primitive nucleus are formed. For example, in a lens of 100 μm diameter (Fig. 3b), one notices that the anterior epithelium is composed of monostratified cuboidal cells, the primitive lens fibers, and the large nucleus.

The pycnotic figures should be noted along with the regions of degeneration which accompany cellular lysis (Fig. 3c), and intranuclear vacuoles. In fact, the rudiment of lens cells encloses a large number of lytic vacuoles (Fig. 3d) or autophagic vacuoles which correspond to the degeneration centers.

FIGURE 5. Electron micrographs of a: the visual cells of the adult (ep represents pigmented epithelium; cv, visual cell; c, centriol; e, ellipsoid); b, the optic nerve (nt, neurotubule; nf, neurofilament); and c, the optic tract (ll represents *lateral lemniscus;* cp, *commissura posterior;* tthy, *tractus tectothalamicus* and *hypothalamicus cruciatus;* and ft, *fasciculus lateralis telencephale*).

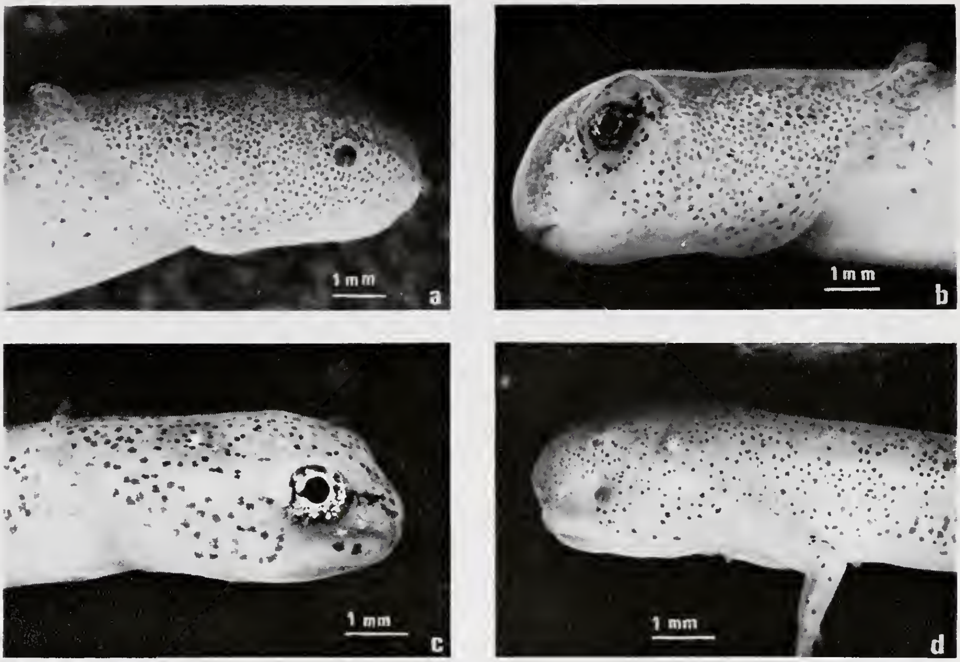


FIGURE 6. Macrophotographs of a: the *Proteus* larva (27 mm length); b, the larva (27 mm length) with a grafted *Euproctus* eye; c, the *Euproctus* larva (19 mm in length); d, the *Euproctus* larva (19 mm length) with a grafted *Proteus* eye.

Conservation of the primitive lumen of the lens, permanence of lytic processes, and the possibility of nucleolar segregation in the cells of the cornea all demonstrate that the involution of the epiblastic derivatives of the eye corresponds to the persistence of a cellular metabolic disturbance (normal but transitory during the ocular development of other vertebrates).

Biometrics can be used to confirm the cytological findings. It is clear that the curves (Fig. 4) represent the growth of the eye and the lens and satisfy respectively the functions: bx^{α} and $bx^{\alpha}\ xe^{-\gamma x}$. The growth pattern of the lens is of the same type as the one for the eye and needs only be differentiated by the complementary term $e^{-\gamma x}$, where γx can be considered as the expression of the involutive phenomenon superimposed upon the normal growth of the lens.

In summary, the slowness of the processes of growth and of involution (along with the modifications which accompany them) suggests that there is in the lens a state of relative equilibrium between the processes of synthesis and degeneration.

Photosensitive retina. The photosensitive retina is composed of a pigmented epithelium which appears poorly developed (Nguyen Legros and Durand, 1974).

The visual cells of *Proteus* (Fig. 5a) appear to be degenerated if compared to those of other Urodela. One can find, however, the elements essential to a photoreceptor: the cell body, the inner segment with mitochondria, and a basal centriole sending ciliary fibrils through a peduncle which supports the outer segment. The existence of a peduncle shows that each very flattened stack of double membrane disks, containing some vesicles and some tubules, corresponds to a rudimentary photoreceptor. The existence of such photoreceptors has been denied by earlier workers.

Neural retina. The disposition of the neural retina is simple; the number of photoreceptive elements, which is about 2000 for an adult *Proteus*, is very low in comparison to 110,000 possessed by *Necturus* whose retina is regarded as poorly developed. The body of the receptor is joined at the base by the fiber of Henlé which terminates by dendritic expansions enclosing synaptic vesicles and synaptic ribbons. The external plexiform layer of *Proteus* is therefore built following the classic pattern. Similarly, the internal plexiform layer includes active zones on a level where the synaptic membranes separate the end buds of the bipolar cells and the dendrites of the ganglionic cells. Relationships between the various categories of nerve cells of the retina appear normal.

Optic tracts. The existence and continuity of the optic tracts have often been contested. The axons of the optic nerve issue from the multipolar cells of the retina, in a tenuous layer joined together in a nervous tract. On their extracranial pathway, the ophthalmic nerve and artery pass above the pterygoid, under the deep ophthalmic branch of the trigeminal nerve and above the cranial trabecula before penetrating the cranium by an optic foramen opening through the orbital lamella of the parietal bone. At this level, the diameter of the optic nerve is from 9–15 μm in the larva and from 10–30 μm in the adult.

The optic nerve enters the brain at the level of the supraoptic nucleus. It is enclosed in a stalk of glial cells, the lumen of which communicates with the optic recess of the third ventricle. The optic nerve is made up of nonmyelinated fibers of very thin diameter (Fig. 5b). For some of them sizes range between 0.10 and 0.60 μm, and they contain neurotubules while others are larger including both neurotubules and neurofilaments. The myelinated fibers are less numerous and their diameter can exceed one micrometer. Their sheaths are composed of ten to twenty lamellae. Some of these fibers terminate in the presynaptic prominence of the anterior accessory optic tract.

The optic fibers are bent back towards the chiasma. From each side of the chiasma a small number of fibers run towards the lateral anterior region of the mesencephalon, making up the axial marginal optic tract (Fig. 5c). The optic tectum of the adult is relatively less developed than that of the young animal and occasionally resembles the degenerated optic region found in cavernicolous fishes.

In summary, the optic tract of the young *Proteus* does not show any break in its continuity between the photoreceptors and the optic tectum. The connection of the organs necessary to transmission of information exists, and this appears to agree with the electrophysiological results of Zener (1971), at least as far as the eye is concerned.

It is evident from the study of the relationships between the eye and the orbit that certain peculiarities of the visual apparatus are related to modifications of its orbital adnexa, such as the elongation of the vessels, muscles and the optic nerve and the displacement of the optic nerve center towards the posterior region of cranium. These are anatomical modifications often connected with the elongation of the head and body in other forms.

EXPERIMENTS ON DEGENERATION OF THE EYE

Subterranean environment and microphthalmy

Lack of pigmentation and ocular degeneration are considered to be two essential characteristics of adaptation of an animal to cavernicolous life. This is true for the lack of pigmentation in *Proteus*. In fact, pigmentation appears at the beginning of development in the absence of all light stimulation. Young larvae maintained in light show melanophores and also yellowish chromatophores. Pigmentation is controlled thereafter by a physiological regulation in relation to conditions of light or darkness present in the environment of the animal. Accordingly, the lack of pigment in *Proteus* appears to be an adaptative character and not a degenerative feature.

The condition is other than that suggested by the term, "ocular degeneration." An adaptative relationship between the darkness underground and the disappearance of the eyes (which have become unnecessary) is accepted by a great number of authors (Gostejeva, 1949). In fact, this opinion only stands on a single experiment, still very debatable, made by Kammerer (1912).

Experiments were designed to find out if the presence of light will actually permit regeneration of eyes. Young specimens of *Proteus* were exposed to the action of daylight for 6.5 years. Others were exposed to artificial light of wavelengths between 600 and 1200 nm (i.e., within the red and infrared range of the spectrum). This continued for the first 10 years of age. After several years, individuals exposed to light become bluish-black. Over the same time, even if the eye is initially relatively well developed, the corneal ectoderm thickens, the lens diminishes in size and eventually disappears.

It is known that at the level of the photoreceptive cells, light exerts a certain action. However at the level of the entire visual apparatus, and within the limits of our experiments, it does not impede at all the manifestation of degenerative processes including the sinking in of the eye within the orbit, the disappearance of the lens, and the thickening of the dermal cornea.

Humoral conditions of ocular involution

The subterranean environment does not seem to have a determinant ontogenetic influence in transplant studies of microphthalmy. This problem was studied in order to test the hypothesis (see, for example, Hawes, 1946), that the eye of *Proteus* results from a stopping of development tied to the neotenic condition of the animal. Xenoplastic grafts have permitted us to follow, after determination of the field, the development of the eye of *Proteus* transplanted to a host in which the eyes develop normally. Conversely, the development of a foreign ocular field was followed in *Proteus* (Fig. 6).

The grafts between *Proteus* and a certain number of other species were rejected (*Triturus* spp.), but transplantations between *Proteus* and either *Pleurodeles waltlii* or *Euproctus asper* gave satisfactory results.

Implants of Proteus. The graft of the ocular field of *Proteus* on the lateral body wall of *Euproctus* or *Pleurodeles* developed only weakly. It is the same for

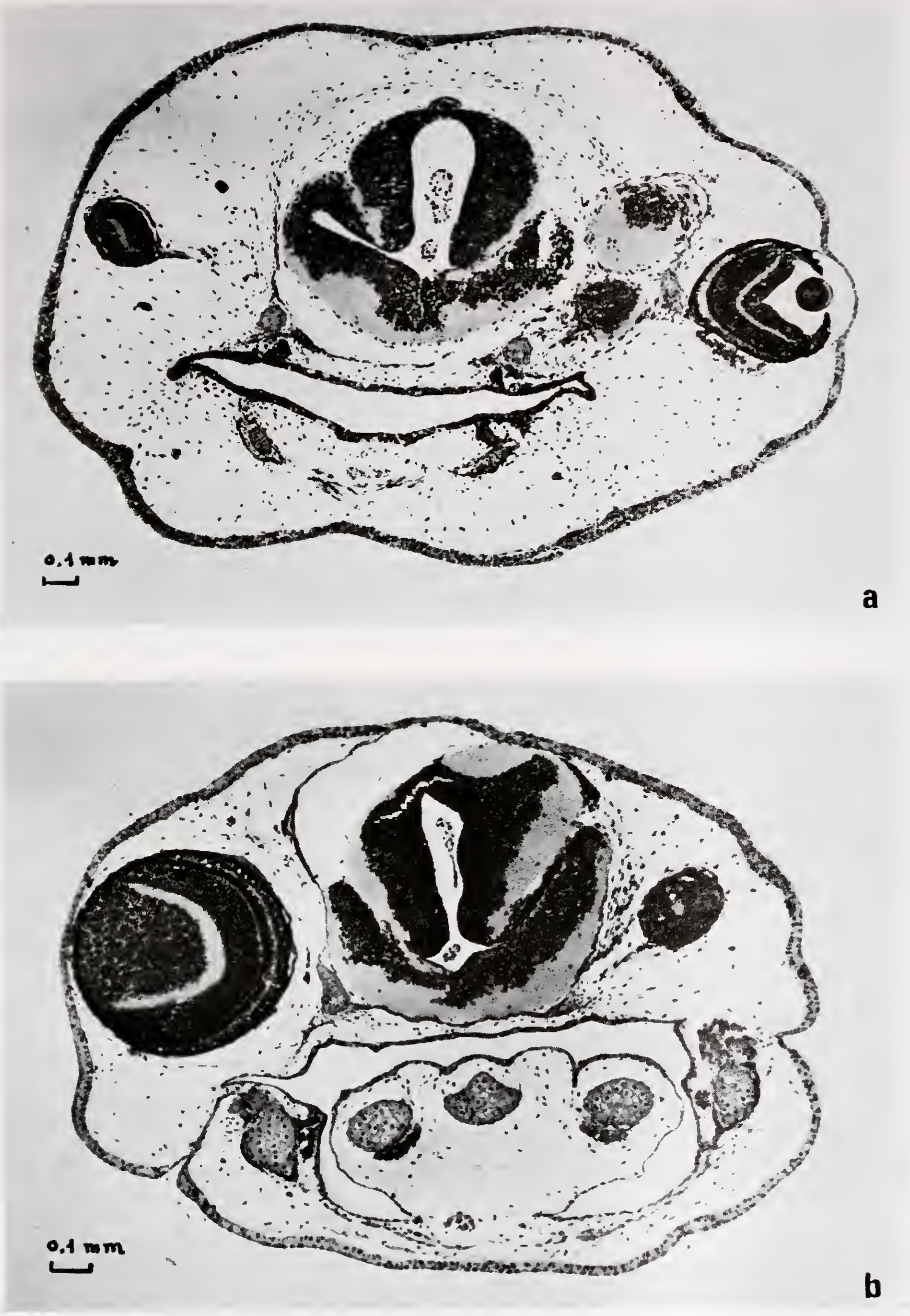


Figure 7. Micrographs of a: the *Euproctus* eye in the orbit of *Proteus* 33 days after transplantation; and b, the *Proteus* eye in the orbit of *Euproctus* 37 days after transplantation.

grafts to the orbits of these animals (Fig. 6d) in which each graft did not go beyond the stage of development normally attained by the eye of *Proteus*. The corresponding histological sections show the weak development of the ocular field of *Proteus* grafted in the orbit of *Euproctus* (Fig. 7b). After transplantation to a foreign host, the autodifferentiation of the ocular anlagen of *Proteus* does not permit it to build a normal ocular apparatus, but only the usual rudimentary eye.

Implants to Proteus. The xenoplastic graft on the lateral body wall of *Proteus* of an ocular field of *Pleurodeles* or *Euproctus,* shows that the field frequently differentiates in an atypical fashion. However, after the homotopic xenoplastic graft of an ocular field of *Pleurodeles* or *Euproctus* on *Proteus,* the graft (Fig. 6b) develops and is pigmented in the same way as it would in the donor orbit (Fig. 7a).

At the end of these experiments, one can say that the grafted eyes develop by autodifferentiation up to and including the time of degeneration. The development of the eye of *Proteus* appears therefore to be independent of the host on which it is grafted.

By a different technique, one can show that the factors responsible for the ocular involution do not have a hormonal origin (thyroxin) as earlier thought. In fact, it is impossible to demonstrate that thyroxin, in solution or by injection has any effect on the development of the eye of *Proteus.* For example, in the eye of animals kept in thyroxin and hydrocortisone solution for five months, with the upper limit of thyroxin being 10 mg/liter, there was only a slight thickening of the general skin.

Mechanism of autodifferentiation

After having shown that the ocular field of *Proteus* develops by autodifferentiation, we tried to demonstrate the mechanism. Reciprocal or cross-transplantation of the neural optic vesicle and its overlying ectoderm allowed the evaluation of the influence of the optic vesicle on the differentiation of a lens or of a cornea. The "inducing influence" of the optic vesicle of *Proteus* operates more slowly and more weakly than that of the optic vesicle of some other Urodela. Conversely, the ectoderm of *Proteus* subjected to a normal inducing action shows a certain "competence" in the differentiation both of a lens and of a cornea. However, this competence is less than that shown by the presumptive ectoderm of either *Pleurodeles* or *Euproctus.*

This can be confirmed by regeneration experiments after ablation of the eye (and particularly of the lens). The morphogenetic ability of the retina is exerted in such cases, later and more slowly in *Proteus.* From this we can conclude that the regulation of organogenesis of the eye unfolds as in the general case, but the reactions of the tissues are specifically weaker and slower than those in the epigeous Urodela.

DISCUSSION

It is worth remembering how much the problem of ocular degeneration of *Proteus* has interested generations of naturalists. Many regard this degeneration as a secondary adaptation to the subterranean environment; in contrast, others regard it as a neotenic developmental character or as an earlier preadaptation to a cavernicolous life. One recent hypothesis is that of Hawes (1946), who considered the ocular reduction of *Proteus* as being dependent on a neotenic preadaptation secondarily reinforced by a subterranean life. In fact, it is very difficult to deduce any phylogenetic evolution for the ocular apparatus of *Proteus,* because the ontogeny of *Proteus* is such that ocular reduction does not depend upon these factors. However, all the anatomical and experimental evidence confirms that the determining factors of the regression are genetic.

The numerous convergences of form between *Proteus* and the other microphthalmic vertebrates such as *Typhlomolge* (= *Eurycea rathbuni* from the caves of Texas), suggest that they result from parallel eye evolution. If one compares the ocular reduction of *Proteus* to that observed in the case of cavernicolous fishes or other Urodela, one can get a clearer idea of the structure and development of these rudimentary eyes. The general pattern involves: slowness of development, instability of the embryonic eye ectoblastic derivatives, and synchrony of constructive with destructive ocular processes. The destructive events involve the cornea and lens initially, but can often lead to the apparent disappearance of the eye in the adult animal.

In the course of this involution, the processes of differentiation to cornea are less important than those of differentiation to teguments. The lens is greatly affected by persistence of lytic processes, which are stronger than those of normal development. The secondary degeneration affects the pigmented epithelium and the photoreceptors, with certain disorganization of the retinal structures and of the optic tractus and tectum.

The usual term "degenerate eye" is misleading. The rudimentary eyes in *Proteus,* and probably in other cave vertebrates, result from specific development and are to be considered as produced by a disturbance of normal ontogenic processes and of cellular metabolism.

The writer wishes to express his sincere thanks to Professor Norton Rubinstein and to the editorial office of the Biological Bulletin for their contributions to translation of this manuscript.

SUMMARY

The anatomy and development of the eye of *Proteus anguinus* are described. The relationships between organogenesis of the eye in embryos and larva and its involution in the young and the adult are discussed.

The availability (in breeding cultures) of a significant number of *Proteus* embryos (which are normally rare) allowed experimental analysis of the effects of light, xenoplastic differentiation and thyroid hormones on the development of the eye.

The results of this study suggest that development and involution of the eye of *Proteus* are controlled by genetic factors which are not greatly influenced by environment, and one can, therefore, consider the microphthalmy of *Proteus* as a relict characteristic which is the result of a specific development with disturbance of the normal ontogenic process.

LITERATURE CITED

Durand, J .P., 1971. Recherches sur l'appareil visuel du Protée, *Proteus anguinus* Laurenti, Urodèle hypogé. *Ann. Spelcol.,* **26**: 497–824.

Gostejeva, M. N., 1949. Nouvelles données sur la réduction des yeux chez les Protées. *C. R. Acad. Sci. U. R. S. S.,* **67**: 177–180.

Hawes, R. S., 1946. On the eyes and reactions to light of *Proteus anguinus. Quart. J. Microsc. Sci. N. S.,* **86**: 1–53.

Kammerer, P., 1912. Experimente über fortpflanzung, farbe, augen und körperreduction bei *Proteus anguinus* Laurenti. *Archiv. Entwicklungsmech. Organismen,* **33**: 348–461.

Nguyen Legros, J. and J. P. Durand, 1974. Ultrastructure de l'épithélium pigmentaire rétinien du Protée, Urodèle cavernicole. Relations fonctionnelles avec les photorécepteurs. *Ann. Speleol.,* **4**: 671–674.

Vandel, A., 1966. Le Protée et sa place dans l'embranchement des Vertebrés. *Bull. Soc. Zool. Fr.,* **91**: 171–178.

Vandel, A., J. P. Durand and M. Bouillon, 1964. Observations sur le développement du Protée, *Proteus anguinus* Laurenti (Batraciens, Urodèles). *C. R. Hebd. Séanc. Acad. Sci. Paris,* **259**: 4801–4804.

Vandel, A. and J. P. Durand, 1970. Le cycle vital du Protée. *C. R. Hebd. Séanc. Acad. Sci. Paris,* **270**: 2699–2701.

Zener, B., 1971. The light sensitivity of the cave salamander *Proteus anguinus* Laur. *Periodicum biologorum, Soc. Sci. Natur. Croatica,* **73A**: 16.

Reference: *Biol. Bull.* **151**: 467–477. (December, 1976)

COAGULATION IN THE CRAYFISH, *ASTACUS LEPTODACTYLUS*: ATTEMPTS TO IDENTIFY A FIBRINOGEN-LIKE FACTOR IN THE HEMOLYMPH

MICHELE DURLIAT AND ROGER VRANCKX[1]

Océanographie biologique, Bâtiment A, Université Pierre et Marie Curie,
4 place Jussieu, 75230 Paris Cedex 05, France

Occurrence of plasmatic clottable protein is one of the most controversial problems of decapod serology. Data are often inconsistent and vary according to the species under consideration. Previous electrophoresis (Durliat, 1974) carried out on crayfish serum and plasma show in the plasma the presence of an additional component which displays a role in coagulation, since it disappears after gelling of the blood. This additional fraction is relatable to the plasmatic clottable extract obtained by the procedures of Duchâteau and Florkin (1954) and Stewart, Dingle and Odense (1966). It is exciting to think that crayfish blood contains a fibrinogen-like protein analogous to that of *Panulirus interruptus* (Fuller and Doolittle, 1971a, b; Doolittle and Fuller, 1972; Tyler and Scheer, 1945), *Homarus americanus* (Stewart *et al.,* 1966) and *Homarus* sp. (Duchâteau and Florkin, 1954).

The work reported here is an attempt to isolate from *A. leptodactylus* hemolymph a plasma protein which participates in clotting and to determine some of its properties.

MATERIALS AND METHODS

Serum and plasma preparation

Serum and plasma pools were obtained from 60 crayfish of both sexes, in a premolt stage (D0 to D3). Hemolymph was withdrawn from each crayfish's pereiopod sinus, then transferred into three tubes. Two samples were immediately and thoroughly mixed with an anticoagulant, a 10% sodium citrate or 0.1 M potassium oxalate solution. Proportions used were one part of anticoagulant for nine parts of hemolymph. This procedure prevented the clumping but not the breakdown of the cells. Plasma was collected after a 4° C centrifugation at 5000 rpm for 20 minutes. The precipitate was discarded and supernatant solution was retained. To obtain serum, hemolymph was allowed to form a nonretracting firm clot in a tube. *Astacus leptodactylus* is a decapod belonging to the coagulation C group (Tait and Gunn, 1918). In this clot, agglutination of hemocytes is insignificant but the gelling of plasma is the most important process. The clot was broken up with a stirring rod and centrifuged also at 5000 rpm. The supernatant was collected and the remaining coagulum discarded.

---

[1] Present address: Atherosclerose U 32, I.N.S.E.R.M., Hôpital H. Mondor, Avenue de Lattre de Tassigny, 94000 Créteil, France.

However another method (Durliat and Vranckx, 1976) of preserving the hemolymph from clotting without damaging the cells gives the same results. This method follows that given by Tyson and Jenkin, 1974, with slight modifications. Crayfish were injected *via* the ventral haemal sinus with 2 ml of 0.25% cysteine hydrochloride in physiological saline containing 20 units of preservative heparin/ml at pH 6.2. After three minutes the animals were bled from the ventral sinus using a syringe containing 1 ml of 0.25% cysteine hydrochloride and 20 units of heparin/ml. The cysteine hydrochloride prevents the hemolymph from clotting, without damaging the cells. Heparin, while not preventing clotting, appears to prevent the clumping phenomenon. The contents of the syringe were, after removing the needle, gently emptied into an ice-cold tube and centrifuged to 3000 rpm for five minutes. Following centrifugation, the cells were resuspended in ice-cold van Harrevald's medium at pH 7.2 containing 20 units of heparin/ml. The supernatant or plasma without cells was retained.

Titration of hemolymph constituents

Measurements were made on Technicon. Total protein rate was detected by the method of Lowry, Rosebrough, Farr and Randall (1951), and amounts of triglycerids, phospholipids, cholesterol, uric acid and glucose of serum and plasma pools were determined.

Plasmatic clottable protein extractions

Salting out procedures, as used by Duchâteau and Florkin (1954) and then Stewart *et al.* (1966) on the blood of *Homarus* sp., were applied to freshly withdrawn pools of citrated or oxalated hemolymphs.

The clottable solution was dialysed against four changes of distilled water for 12 hours to eliminate $SO_4(NH_4)_2$ which would inhibit the gelling, before setting up the coagulation tests.

Coagulation assays

These were performed on complete oxalated or citrated hemolymph (anticoagulant solutions were prepared in distilled water), on serum discarded from the gel fraction after coagulation and on "clottable protein" solutions obtained by the salting out method. Complete hemolymph (500 μl) is clotted with a mixture of 50 μl 0.1 M $CaCl_2$ and coagulable protein extract (200 μl) to which is added 50 μl 0.1 M $CaCl_2$ and 50 μl of cellular extract prepared by the method of Stewart *et al.* (1966).

Electrophoretic analysis

Disc electrophoresis was performed on 6% acrylamide gels according to the method of Ornstein (1964), with a tris glycine buffer pH 8.5 at 2 mAmp per tube for two hours on a concave polyacrylamide gel gradient (gradipore) with a tris borate buffer, with or without EDTA, at pH 8.2. Migrations were carried out for 24 hours at 60 V (15 mAmp). Proteins were stained by black amido 10 B during one hour and faded by 7% acetic acid. The cupric fractions were stained by rubeanic acid (Decleir, 1961).

Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis procedures were essentially those described by Schwartz, Pizzo, Hill and McKee (1971), in continuous buffer. All preparations were incubated at 37° C for 24 hours in 0.04 M monodiphosphate buffer pH 7.1 containing urea 3% SDS, in the presence and absence of 3% mercaptoethanol. Gels were run in phosphate buffer containing 0.1% SDS for 19 hours at 15 mAmp and 30 V; they were stained with Coomassie blue after fixing in 10% acetic acid.

Immunologic analysis

Animal immunization. Antisera against serum, plasma and clottable protein were prepared in the I.R.S.C. of Villejuif, using for each solution two "Géant des Flandres" rabbits. They were inoculated with a pool from whole citrated hemolymphs of five crayfish of both sexes, according to the following method of Kabat modified by Burtin (personal communication).

Each rabbit received, in subcutaneous injections into the hind paws, a mixture of 0.5 ml antigens plus 0.5 ml Freund's adjuvant. Two weeks later, a series of three inoculations were effected during three consecutive days with 0.5 ml mixture containing: 0.5 ml antigens, 0.5 ml physiological saline and 0.5 ml 1% ammonium alum. The first injection was made subcutaneously, the next two intravenously. The products were tested, five days after the last inoculation, and the ultimate puncture was rechecked a week later. Antiplasma was obtained from a first pool of hemolymphs during the months of September through October, and antiserum from a second pool during the month of February.

Immunoelectrophoresis. Immunosera were tested by the double diffusion procedure (Ouchterlony, 1967) and immunoelectrophoresis with 1% agarose indubiose A37 gels. Techniques from Grabar and Burtin (1960), Laurell (1966) and Clarke and Freeman (1967) modified by Krøll (1973) were successfully employed; the migrations were carried out in 0.3 M tris barbital sodium barbital buffer pH 8.8. In the qualitative analysis, according to Grabar and Burtin's procedure, electrophoresis was run for 1.5 hr at 160 V and 15 mAmp; quantitative studies in Laurell required a 16 hour migration at 160 V and 18 mAmp and tandem-crossed immunoelectrophoreses were made in the first dimension for 25 hr at 160 V and 15 mAmp and during 18 hr with 130 V and 12 mAmp in the second dimension. Sera and plasmas were diluted by half. Pure solutions of plasmatic clottable extract were used.

RESULTS

Titration of serum and plasma constituents

The results (Table I) are only available for the hemolymph of studied animals in premolt stages. A significant difference was noted between serum and plasma in the total protein content: 7 g liter. It might be argued that this divergence results from the mobilization of the coagulable plasmatic protein at the time of hemolymph gelling.

Electrophoretic analysis

Serum and plasma. Electrophoretic differences between serum and plasma from the same pool of hemolymphs were always observed. On 6% polyacrylamide gels, earlier experiments showed (in the plasma) an additional band (number 1) stopped at the cathodic part of the gel and another one (number 5) which is much more important than in the serum (Durliat, Vranckx, Herberts and Lachaise, 1975). On continuous gradient gels, the plasmatic clottable fraction might show both forms: either as a band of heavy molecular weight or as a multiple banding pattern (Fig. 1). These bands move into the gel approximately between the α_2 macroglobulin and the β lipoprotein position. Because of the logarithmic aspect of the banding pattern, it seems that these different fractions represent the polymeric states of a single subunit.

Specific determination of copper (rubeanic acid) shows identical cuproproteinaceous fractions in serum and plasma. Therefore the clottable factor which disappears or is very faint in the serum probably represents a protein displaying a role in the coagulation processes.

Plasmatic clottable protein. In the same conditions, electrophoresis of different fractions precipitated by ammonium sulfate, showed that the second precipitate (45% saturation) effectively presented an important enrichment in a component corresponding either to the supplementary plasmatic band or to the multiple banding pattern and shadows of the other fractions. The impurities can be partly eliminated after filtration on an agarose A 1.5 column. It was noted that the first precipitate obtained by the salting out procedure (30% saturation) also contained small quantities of clottable protein.

SDS electrophoresis (Fig. 2). Differences between serum and plasma persisted after incubation with SDS, which abolishes all variations proceeding from electrical charges. The clottable extract occurred as a fraction in the cathodic position.

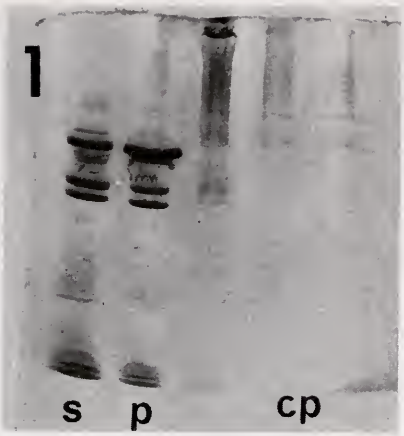
Clotting studies

Whole hemolymph. One ml of whole oxalated hemolymph with the addition of 100 µl 0.1 M calcium chloride clotted very strongly in 15 minutes. The assays performed on 37 animals were all successful. However, when the same experiments were performed on citrated hemolymph, using sodium citrate solution pre-

TABLE 1

Titration of serum and plasma constituents.

	Serum g/liter	Plasma g/liter
Total protein content	39.5	46.4
Triglycerids	0.20	0.22
Phospholipids	0.80	0.88
Cholesterol	0.40	0.40
Uric acid	0.20	0.20
Glucose	0.45	0.45



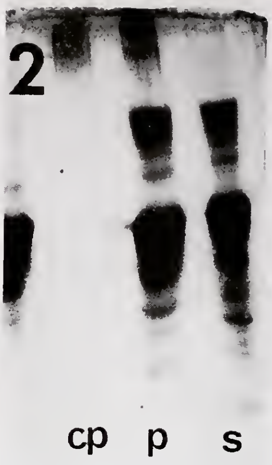


FIGURE 1. Electrophoresis on continuous gradient gels of the crayfish hemolymph, in tris borate EDTA buffer pH 8.2. Serum is represented by (s); plasma (p); and clottable protein (cp).

FIGURE 2. Sodium dodecyl sulfate electrophoresis after incubation in SDS and urea presence. Gel was run in phosphate buffer containing 0.1% SDS for 15 hours with 15 mAmp and 30 V. Symbols are as in Figure 1.

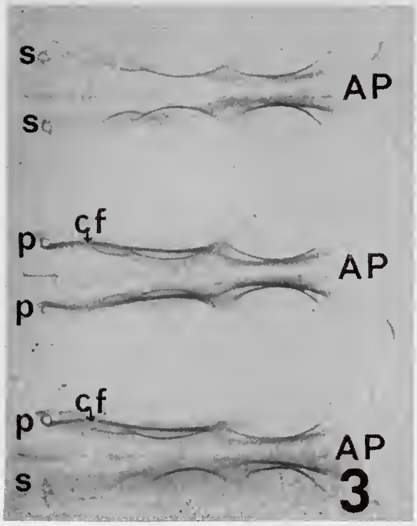
pared in distilled water, no clot was formed. It seems that this anti-coagulant chelates the calcium necessary for the transformation of the clottable protein into gel and also irreversibly blocks the conversion. The solutions of sodium citrate or potassium oxalate do not block the hemolymph clotting in the same way. With sodium citrate, univalent ions were necessary to permit the reversibility of this reaction. It was noted that the pH values of sodium citrate in distilled water or in physiological saline were almost identical.

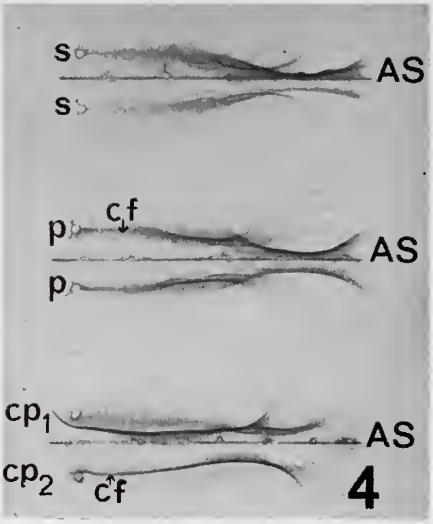
However when univalent ions were added in the citrate solution, before the mixture with hemolymph, the clotting appeared easily (0.5 ml of blood was withdrawn on 100 μl 0.1 M sodium citrate + 100 μl 0.1 M NaCl or KCl and clotted in 15 minutes following the addition of 100 μl 0.1 M $CaCl_2$).

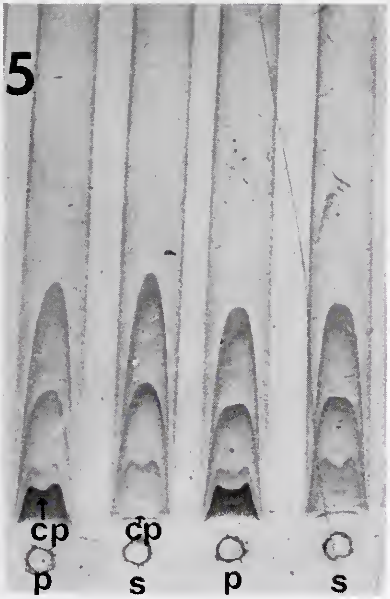
Plasmatic clottable protein. When obtained from oxalated plasma, it clotted very strongly within 10 minutes (0.1 ml clottable fraction + 50 μl 0.1 M $CaCl_2$ + 50 μl cellular extract); but it failed to elicit a clotting response when it was extracted from citrated blood, by the same salting out procedure. However, with a citrated plasma containing Na^+Cl^- ions, the extraction furnished a solution which clotted perfectly.

On the polyacrylamide gradient gels, no differences were noted between the electrophoretic patterns of active and inactive preparations. Clotting tests performed with clottable protein reconstituted after storage in $(NH_4)_2\ SO_4$ were uniformly negative, but freezing seems to have no effect on its clottability.

Serum. The same assays were carried out with the serum to verify the presence or absence of coagulable proteins still available. Reclotting was not possible.







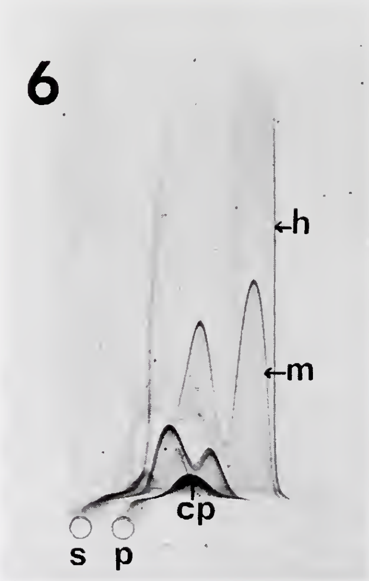


FIGURE 3. Immunoelectrophoresis against an antiplasma of crayfish. Migration for 1.5 hr with 160 V and 15 mAmp in tris barbital sodium barbital buffer pH 8.8. AP shows antiplasma; cf represents the precipitate line of clottable factor. Other symbols are as in Figure 1.

FIGURE 4. Immunoelectrophoresis against an antiserum of crayfish. Migration for 1.5 hr with 160 V and 15 mAmp in tris barbital sodium barbital buffer pH 8.8. Clottable protein extract before filtration (cp1) and after filtration (cp2) is shown on agarose column; AS shows antiserum. Other symbols are as in Figure 1 and Figure 3.

FIGURE 5. Immunoelectrophoresis according to Laurell. Symbols are as in Figure 1; and the antigens result from amounts of plasma and serum in each series from left to right

Immunologic analysis

When serum and plasma were tested against an antiplasma, a distinct precipitate line was present in the plasma (Fig. 3), but appeared only as a shadow in the serum. Clottable extract showed the same precipitate line when tested against this antiplasma. It was noted that this isolated component was not exempt from minor contaminants, because two other arcs were present. One of these other fractions appeared to be hemocyanin. By varying the antigen/antibody ratio, and especially in a large excess of antibody, it was possible to obtain the same picture against an antiserum (Fig. 4).

By quantitative immunoelectrophoresis (Fig. 5) both serum and plasma gave the same number of peaks. When a protein is present in the same amounts in samples, peaks of the same height are observed. This was the case with all but one of the proteins of both serum and plasma. On Figure 5 the lowest peak, assumed to be the clottable factor, was well represented in the plasma but was insignificant in the serum.

Immunological identities between the different proteins of both serum and plasma were evidenced by tandem-crossed immunoelectrophoresis (Clarke and Freeman, 1967). All proteins gave double-headed peaks except the clottable factor (Fig. 6).

Furthermore, by Ouchterlony diffusions, a protein with a complete antigenic identity appeared in the serum, plasma and clottable fraction (Fig. 7). There is still clottable protein in the serum because a single clotting process does not remove all the plasmatic coagulable protein. This phenomenon was clarified in the spiny lobster *Jasus paulensis,* in which serum was reclotted several times (Durliat and Vranckx, in preparation). The reclotting of *Astacus* serum was not possible because the amount of the remaining clottable protein was too low. This protein disappeared in the sample of serum when it was absorbed with an anticlottable extract.

Discussion

Evidenced in *Homarus* sp. (Duchâteau and Florkin, 1954), *Homarus americanus* (Stewart *et al.,* 1966), *Callinectes sapidus* and some other decapods (Manwell and Baker, 1963), and *Panulirus interruptus* (Fuller and Doolittle, 1971 a, b; Doolittle and Fuller, 1972), a plasmatic "fibrinogen"-like factor has not been found in *Gecarcinus lateralis* (Stutman and Dolliver, 1968), *Cancer irroratus, Cancer*

---

of 30 μg and 25 μg. For the antibody an agarose gel containing 1% rabbit serum anticrayfish plasma (2 μl/cm^2) is used. Migration run for 16 hours with 160 V and 18 mAmp in tris barbital sodium barbital buffer pH 8.8 at 4° C. Note that the same number of peaks occur in serum and plasma, but that the clottable factor very important in plasma was insignificant in serum.

FIGURE 6. Tandem crossed immunoelectrophoresis. Premolt protein is represented by m; hemocyanin by h. Other symbols are as in Figure 1. An antibody of agarose gel containing 6% rabbit serum anticrayfish plasma (10 μl/cm^2) is used. Antigens involve amounts of serum and plasma of 100 μg. Migrations run in first dimension: 2.5 hr with 160 V and 15 mAmp; second dimension: 18 hours with 130 V and 12 mAmp, in tris barbital sodium barbital buffer pH, 8.8 at 4° C. All proteins give double-headed peaks, except the clottable factor.

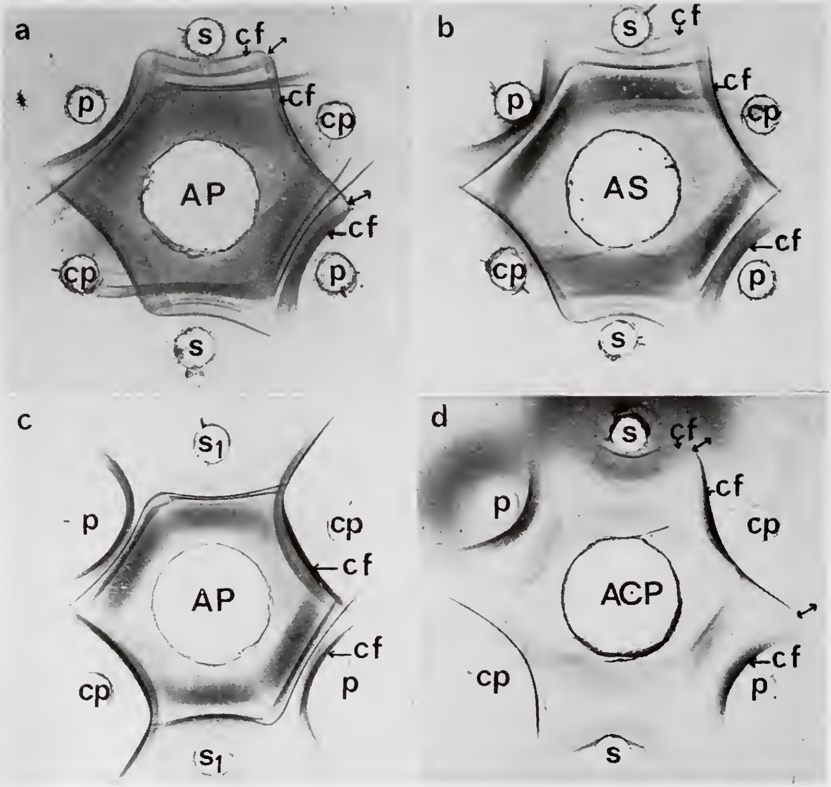


FIGURE 7. Ouchterlony studies with as antibodies: antiserum (100 μl) represented by AS; antiplasma (100 μl) by AP; and anticlottable protein (2.5 μl) by ACP. For the antigens: serum absorbed with anticlottable protein is represented by S1; precipitate line of clottable factor by cf. Other symbols are as in Figure 1. The clottable factor of serum, plasma and coagulable extract gives a continuous precipitate line showing a complete identity (↔). In Figure 7c this precipitate line is not evidenced against S1 because remaining clottable factor was absorbed against anticoagulable protein. On each immunodiffusion, two different amounts of S, P and cp are loaded so that variation in density of precipitate line are recorded.

borealis, Hyas coarctatus (Stewart and Dingle, 1968), nor *Oronectes virilis* (Wood and Karpawich, 1972). More exactly, it was not detected by electrophoretic analysis. In the last cases plasma and serum proteins were, according to these authors, almost identical.

It is possible that the explanation of this difference lies in the quantities of available plasmatic clottable protein which, when these quantities are too low, cannot be detected by electrophoretic analysis. For instance, in *Gecarcinus lateralis,* plasma and serum electrophoregrams are similar, but hemolymph microscopic observations show the development of fibrin-like strands (Stutman and Dolliver, 1968).

Moreover, other observations show that the level of plasmatic clottable factor depends not only on the species of the examined animal, but also on its physiological state (intermolt stage, captivity, pathology). Therefore, it is likely that the conjunction of all these factors, internal and external, may explain the great variability of data already reported in the literature on plasmatic clottable protein.

The heterogeneous aspect of this coagulable plasmatic protein was noted; it occurs either as a fraction of molecular weight 3,200,000 or as a multiple banding pattern. However in *Panulirus interruptus*, Doolittle and Fuller (1972) have revealed with SDS and mercaptoethanol electrophoresis that the clottable factor appears as a monomer unit with a molecular weight of 400,000 and a dimer of 800,000. Other observations (Durliat *et al.*, 1975) seem to indicate that in *Astacus leptodactylus* the clottable factor undergoes a polymerization as time goes on.

Our recent experiments have demonstrated that the monomer has its origin in the hemocytes. This protein is also present as an integral part of the plasma before any leakage from blood cells (Durliat and Vranckx, 1976). This clotting process seems to be different from that in the horseshoe crab *Limulus polyphemus*, which consists only in the conversion of a clottable protein located entirely in the hemocytes into a gel by an enzymatic system (Levin and Bang, 1968; Young, Levin and Prendergast, 1972).

On the other hand, lacking knowledge of the structure of this protein, presence of traces of clottable-related fractions in the serum may be explained either as a fibrinopeptid resulting from degradation of the native protein or as the rest of this gelling protein which has not clotted. However, Fuller and Doolittle (1971b) show an absence of the "fibrinolysis" process as it occurs in the vertebrates. In our work, Ouchterlony diffusions do not seem to show any difference between the clottable fractions of various origins. One may postulate that during coagulation all the clottable fractions are not needed nor used and that the remainder found in the serum may still induce specific antibody formations in the rabbit. This explains the fact that the same kinds of immunoelectrophoresis are observed when plasma is tested against either antiplasma or antiserum. Thus, these antibodies exist together in the antiserum and antiplasma, but the precipitation reaction is obvious only when the stoichiometric ratio is suitable.

Ionic requirements are only reported but not at all understood. It was established by reclotting assays that monovalent cation was needed to be added to the citrated solution before mixing with the hemolymph rather than to the oxalated solution. Is the clottable protein or the clotting enzyme irreversibly disturbed by the citrate in the absence of a monovalent cation?

On the other hand, the tandem-crossed immunoelectrophoresis carried out against an antiplasma (Fig. 6, m) shows a protein existing in serum and plasma, but disappearing when the samples are tested against an antiserum. Antiplasma has been prepared in September from hemolymphs of premolt stage animals and antiserum in February from crayfish blood in intermolt (C4); one can postulate that it is a cuticular protein becoming overt in the crayfish approaching the molt. This supposition has been confirmed with antiserum and antiplasma obtained from same hemolymph pool from animals in different intermolt stages (Vranckx and Durliat, 1976).

In summary, the tests performed on the hemolymph of *Astacus leptodactylus* demonstrate a coagulable plasmatic protein, analogous to a "fibrinogen"-like fraction, of large molecular weight and high antigenicity.

The authors thank Françis Humbert for technical assistance. We thank Dr. J. L. Beaumont for providing many laboratory facilities and for valuable discussions.

SUMMARY

1. A series of tests were conducted to determine whether or not the hemolymph of the crayfish *Astacus leptodactylus* contains a plasma coagulation factor.

2. The total protein amount is higher in the plasma than in the serum.

3. Serum and plasma do not exhibit similar electrophoretic banding patterns. Plasma contains one band or a series of supplementary fractions with a high molecular weight.

4. Electrophoregrams of plasmatic clottable extract, obtained by the classical methods employed in crustacean serology, show a main fraction or a series of polymers with the same electrophoretic behavior as the additional fractions seen on the plasma pattern.

5. This solution clots when treated with $CaCl_2$ and a cellular extract.

6. Immunoelectrophoresis demonstrates the presence of a clottable protein precipitate line in plasma, but this protein also gives a very faint similar line in serum.

LITERATURE CITED

Clarke, H. G. M., and T. Freeman, 1967. A quantitative immunoelectrophoresis method (Laurell electrophoresis). *Prot. Biol. Fluids,* **14**: 503–509.

Decleir, W., 1961. The localization of copper in agar gel electrophoresis patterns of crustacean blood. *Naturwissenschaften,* **48**: 102–103.

Doolittle, R. F., and G. M. Fuller, 1972. Sodium dodecyl sulfate polyacrylamide gel electrophoresis studies on lobster fibrinogen and fibrin. *Biochem. Biophys. Acta,* **263**: 805–809.

Duchâteau, G., and M. Florkin, 1954. La coagulation du sang des Arthropodes. IV. Sur le fibrinogène et sur la "coaguline" musculaire du Homard. *Bull. Soc. Chim. Biol.,* **36**: 295–305.

Durliat, M., 1974. Etude de la coagulation de l'hémolymphe chez les Crustacés Décapodes. Analyse électrophorétique des protéines du sérum et du plasma d'Ecrevisse (*Astacus leptodactylus*). *C. R. Hebd. Séanc. Acad. Sci. Paris Ser. D,* **278**: 473–476.

Durliat, M., and R. Vranckx, 1976. Analyse des protéines hémocytaires d'*Astracus leptodactylus. C. R. Hebd. Séanc. Acad. Sci. Paris Sér. D,* in press.

Durliat, M., R. Vranckx, C. Herberts, and F. Lachaise, 1975. Effets de la congélation sur la séparation electrophorétique des protéines de l'hémolymphe de quelques Arthropodes. *C. R. Séanc Soc. Biol.,* **169**: 862–867.

Fuller, G. M., and R. F. Doolittle, 1971a. Studies of invertebrate fibrinogen. I. Purification and characterization of fibrinogen from spiny lobster. *Biochemistry,* **10**: 1305–1310.

Fuller, G. M., and R. F. Doolittle, 1971b. Studies of invertebrate fibrinogen. II. Transformation of lobster fibrinogen into fibrin. *Biochemistry,* **10**: 1311–1315.

Grabar, P., and P. Burtin, 1960. Analyse immuno-électrophorétique. Ses applications aux liquides biologiques humains. Masson et Cie, Paris.

Krøll, J., 1973. Quantitative immunoelectrophoresis. 4. Tandem crossed immunoelectrophoresis. *Scand. J. Immunology, Suppl.,* **1**: 57–59.

Laurell, C. B., 1966. Quantitative estimation of proteins by electrophoresis in agarose gel containing antibodies. *Anal. Biochem.,* **15**: 45–52.

Levin, J., and F. B. Bang, 1968. Clottable protein in *Limulus:* its localization and the kinetics of its coagulation by endotoxin. *Thromb. Diath. Heamorrh.,* **19**: 186-197.

Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall, 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.,* **193**: 265–275.

Manwell, C., and C. M. A. Baker, 1963. Starch gel electrophoresis of sera from some marine Arthropods: studies on the heterogeneity of hemocyanin and on a "ceruloplasmin like protein." *Comp. Biochem. Physiol.,* **8**: 193–208.

Ornstein, L., 1964. Disc electrophoresis. I. Background and theory. *Ann. N. Y. Acad. Sci.,* 121: 321-349.

Ouchterlony, O., 1967. Immunodiffusion and immunoelectrophoresis. Pages 655–706 in D. M. Weir, Ed., *Handbook of Experimental Immunology.* Blackwell, Oxford and Edinburgh.

Schwartz, M. L., S. V. Pizzo, R. L. Hill, and P. A. McKee, 1971. The effect of fibrin-stabilizing factor on the subunit structure of human fibrin. *J. Clin. Invest.,* **50**: 1506–1513.

Stewart, J. E., and J. R. Dingle, 1968. Characteristics of hemolymph of *Cancer irroratus, Cancer borealis* and *Hyas coarctatus. J. Fish. Res. Board Can.,* **25**: 607-610.

Stewart, J. E., J. R. Dingle, and P. H. Odense, 1966. Constituents of the hemolymph of the lobster *Homarus americanus* Milne Edwards. *Can. J. Biochem.,* **44**: 1447–1459.

Stutman, L. J., and M. Dolliver, 1969. Mechanism of coagulation in *Gecarcinus lateralis. Ann. Zool.,* **8**: 481–489.

Tait, J., and D. J. Gunn, 1918. The blood of *Astacus fluviatilis:* a study in crustacean blood, with special reference to coagulation and phagocytosis. *Quart. J. Exp. Physiol.,* **12**: 35 80.

Tyler, A., and B. T. Scheer, 1945. Natural heteroagglutinins in the serum of the spiny lobster, *Panulirus interruptus.* II. Chemical and antigenic relation to blood proteins. *Biol. Bull.,* **89**: 193–200.

Tyson, C. J., and C. R. Jenkin, 1974. Phagocytosis of bacteria *in vitro* by haemocytes from the crayfish (*Parachaeraps bicarinatus*). *Aust. J. Exp. Biol. Med. Sci.,* **52**: 341–348.

Vranckx, R., and M. Durliat, 1976. Analyse immunologique des variations des protéines de l'hémolymphe d'*Astacus leptodactylus* pendant la prémue. *C. R. Hebd. Séanc. Acad. Sci. Paris Ser. D.,* 282: 1305-1308.

Wood, P. J., and P. P. Karpawich, 1972. Studies of the coagulation process in the crayfish, *Orconectes virilis:* attempts to identify a fibrinogen-like factor. *Comp. Biochem. Physiol.,* **42**: 41–48.

Young, N. S., J. Levin, and R. A. Prendergast, 1972. An invertebrate coagulation system activated by endotoxin. Evidence for enzymatic mediation. *J. Clin. Invest.,* **51**: 1790.

Reference: *Biol. Bull.* **151**: 478–488. (December, 1976)

GENETICS AND ASEXUAL REPRODUCTION OF THE SEA ANEMONE *METRIDIUM SENILE*

RICHARD J. HOFFMANN

Department of Life Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania 15260; and Woods Hole Oceanographic Institution, Woods Hole, Massachusetts 02543

One problem in gaining an understanding of the mechanisms of natural selection centers around the difficulty in observing the dynamics of genetic processes. There are only a few studies where natural selection has been observed in the process of altering the genetic composition of natural populations. Probably the most important reason for this lack of information is one of time. Most population samples taken for genetic studies are collected only once; rather few studies have attempted to follow changes in gene frequencies with time. The classic studies of chromosomal polymorphism in *Drosophila* by Dobzhansky and co-workers (see Dobzhansky, 1970) are an outstanding exception.

There have been attempts to add a temporal dimension to genetic studies by indirect means. For example, it often is possible to gain approximate indication of the relative ages of organisms by their sizes. Boyer (1974) for *Mytilus edulis* and Koehn, Turano, and Mitton (1973) for *Modiolus demissus* have shown differences in gene frequency with size, an indicator of age in bivalves. Tracey, Bellet, and Gravem (1975) have shown similar differences in *Mytilus californianus*. While differential survival of genotypes is one potentially important component of fitness, it may not imply differential reproduction of genotypes. In addition, studies like these, especially involving animals with widely dispersed pelagic larvae, cannot rule out the possibility that differences in gene frequency with size class are the result of derivation of the size classes from different founder populations with different gene frequencies. In fact, Tracey *et al.* (1975) interpret their data on *M. californianus* as a product of this kind of population structure. Parallel variation between ecologically similar species, such as that documented by Koehn and Mitton (1972), adding differences between size classes, would be more convincing.

Metridium senile (L.) is a conspicuous sea anemone occupying rocks and pilings in protected and semiprotected habitats along the New England coast. The species exhibits color polymorphism, with individuals ranging from white to brown and red; the colors are produced by combinations of melanin and carotenoids (Fox and Pantin, 1941; Fox, Crozier and Smith, 1967). Sexual reproduction is a feature of the life cycle with eggs and sperm shed freely into the surrounding medium (Gemmill, 1920). In the Woods Hole region spawning occurs during the summer months, and the species is dioecious (Costello, Davidson, Eggers, Fox, and Henley, 1957, cited in Campbell, 1974). Asexual reproduction by pedal laceration is also prominent, commonly leading to large aggregations of identically colored individuals (also Torry, 1902).

Asexual reproduction, coupled with the sessile existence of the adult, are special features of the life cycle of *M. senile* which make it feasible to add a temporal dimension to genetic studies of the species, even when sampling is possible at only a single time. Since long distance dispersal of new, asexually produced polyps is unlikely, and since each clone is produced from a single settling planula larva, it is possible to measure potential differential proliferation of genotypes under local natural conditions. Genotype distributions of successful planulae can be determined, since they will be reflected by the genotypes of clones; and the success of each established anemone can be measured by the numbers of monoclonal individuals that it has produced since settling. Of course, this ignores large portions of the life cycle which are probably also subject to intense selection, most prominently as a result of larval and immediate post-settling mortality. Further, Williams (1975) has recently emphasized the importance of studies on organisms with both sexual and asexual phases. This study takes advantage of the special features of the life cycle of *M. senile* to investigate dynamic aspects of genetic structure during asexual reproduction.

MATERIALS AND METHODS

Methods of collection

Animals were collected from rocks, pilings, and the shells of *Mytilus edulis* by carefully scraping the foot loose with a thin spatula or penknife. They were returned to the laboratory in jars of sea water and were held for electrophoresis on water tables supplied with continuously running sea water.

Electrophoresis

Horizontal starch gels were cast from 13% (W/V) Sigma starch using covers and slot formers. The buffer system was the discontinuous lithium hydroxide system of Selander, Hunt, and Yang (1969). Gels were cast two hours before use, since prolonged aging (*e.g.,* overnight) produced unacceptable gels. Small samples of pedal disk or column of individual polyps were ground in approximately 0.5 ml of 0.1 M tris, pH 7 and centrifuged for 20 minutes at top speed in a clinical centrifuge. About 30 μl of the supernatant were loaded into each sample well, and 350 volts were applied for 5 hours. After electrophoresis, gels were sliced and stained for phosphohexose isomerase (PHI) in the following mixture: 10 mg fructose-6-phosphate, 100 mg $MgCl_2$, 10 mg NADP, 4 mg phenazine methosulfate (PMS), 20 mg MTT tetrazolium, and 20 units glucose-6-phosphate dehydrogenase to 100 ml in 0.1 M tris, pH 8. Substrates and enzymes were purchased from Sigma. Buffer components were standard reagent grade.

Locations

Three locations on Cape Cod, Massachusetts, were chosen for study.

Woods Hole Oceanographic Institution Dock. This location is on the south side of Cape Cod, an area strongly influenced by the warming of the Gulf Stream during the summer. Anemones were collected subtidally on the shells of mussels, which were gathered from an area approximately 1 m^2. No analysis of clonal association (see Barnstable) was attempted, although individuals on a single mussel tended to be of like color, suggesting some asexual reproduction.

Cape Cod Canal. Animals were collected intertidally from rock surfaces and mussel shells found on the north jetty at the eastern end of the canal. This is a relatively high energy environment, due to currents and wave action in the canal. Consequently, animals were found in relatively protected locations, usually in the crevices among the large rocks constituting the jetty. On the first occasion animals were collected without reference to specific station or location with respect to other animals. Although detailed mapping of individual polyps for clonal analysis was technically not feasible because of close working quarters between the rocks, on a subsequent collecting trip animals were kept separate according to the rock from which they were collected to gain some indication of patchiness of distribution of genotypes. There was no appreciable color polymorphism observed at this location; all animals collected were brown.

Barnstable Town Boat Harbor. Barnstable Harbor is a protected bay on the north side of Cape Cod. The town boat harbor is a deep sloping basin lined with large rocks. Anemones were found near the low tide mark attached to these rocks and to dock pilings. Animals were generally large (> 5 cm pedal diameter), and there were sizable associations of identically colored individuals, indicating asexual reproduction. There was also a high frequency of diglyphic animals, a feature produced by asexual reproduction by pedal laceration when the piece pinched off includes a directive mesentery (Hahn, 1905).

Clonal assessments at Barnstable

Initial collections were made from two restricted stations (one or two rocks at each station), and animals collected at the same station were pooled for genetic analysis. During subsequent collections the relative positions of individual polyps were sketched by an assistant as the animals were removed from the substratum. In order to ensure complete sampling of clones, all visible animals at each station were collected. Individuals were kept separate by map location until electrophoresis could be performed.

In this population of *M. senile* it was possible to estimate clonal limits by analyzing the patterns of distribution of colors and PHI genotypes. While the mode of inheritance of color pattern is not understood, it is clear that color in *M. senile* is constant over considerable periods of time, if not the entire life of the polyp; it apparently cannot be modified by diet in spite of direct derivation of carotenoids from food (Fox *et al.*, 1967). The supposition that color is indeed genetic, rather than an environmentally induced character, is further reinforced by the occurrence of large aggregations of identically colored anemones that are often accompanied on the same rock or piling by a group of an entirely different color. A similar situation is observed in *Anthopleura elegantissima* on the Pacific coast (Francis, 1973a). For present purposes color is assumed to be constant for a given clone, and probably genetically determined. By using a combination of color, location, and PHI genotype, clone sizes and distributions were determined from the maps made in the field.

Animals were considered to be members of the same clone if, and only if, they met the following four criteria: if they occurred on the same rock or piling, if they were the same color, if they had the same PHI phenotype, and if they were not entirely separated from a similar group by a large aggregation of a different constitution (*i.e.,* assuming that movement through another clone of closely spaced individuals is unlikely and that separation did not occur prior to the proliferation of the intervening clone). Of course, this also assumes that there is no nonelectrophoretic variation for PHI. In one case, a large individual was assigned to a clone on another closely adjacent rock because it shared an unusual color phenotype (tan with brown freckles on the column) with the larger group on the next rock.

While it is possible that there are some errors in assignment to clones by this method, they should be few, since the probability of joint occurrence of the four conditions is low unless the animals so assigned are indeed monoclonal. (An estimate of the maximum probability of error in assignment can be made as follows. There were a total of nine rocks and pilings examined in the study, so the probability of being on any of them at random is 1/9 or 0.111. The most frequent color at Barnstable is brown, and the random probability of being brown is 0.784 (120/153). The most common PHI genotype is f/s, which occurred with a frequency of 0.516. On these three conditions alone, the joint probability is the product of the independent probabilities, so $P = 0.045$. This being the maximum probability of joint occurrence, it seems certain that $P < 0.05$, overall.) This kind of analysis made possible inferences about relative propensities of genotypes to proliferate, since the original distribution of genotypes of successful planulae is known (each clone being produced by a single planula larva), as is the composition of the clones at the time of sampling.

RESULTS

Three PHI phenotypes were observed from all collecting locations. These patterns consisted of two single banded classes, one designated "fast" on the basis of electrophoretic mobility; the other was designated "slow." The third pheno-

TABLE I

Gene frequencies and zygotic distributions from three localities studied for phosphohexose isomerase variation in Metridium senile, *including χ^2 analysis for goodness of fit to Hardy-Weinberg expectations. Expected numbers shown in parentheses; s.e. = $\sqrt{(pq/2N)}$.*

Location	N	Gene frequency f(±s.e.)	Genotypes			$\chi^2_{[1]}$	P
			f/f	f/s	s/s		
Woods Hole	90	0.789 ± 0.030	53 (56)	36 (30)	1 (4)	3.61	<0.10
Cape Cod Canal (all individuals)	200	0.845 ± 0.018	155 (142.8)	28 (52.4)	17 (4.8)	43.29	<0.001
Cape Cod Canal* (without B-2)	157	0.917 ± 0.016	132 (132.1)	24 (23.8)	1 (1.1)	0.01	<0.95
Barnstable† (clones)	27	0.685 ± 0.063	12 (12.7)	13 (11.7)	2 (2.7)	0.36	<0.70
Barnstable‡ (mapped individuals)	153	0.716 ± 0.026	70 (78.4)	79 (62.3)	4 (12.4)	11.07	<0.001
Barnstable (all individuals)	245	0.678 ± 0.021	93 (112.5)	146 (107)	6 (25.5)	32.50	<0.001

* Canal data analyzed without individuals from station B-2, which had a large number of s/s animals.

† Analysis of clones as the genetic individual. Clones were determined as described in the text.

‡ Barnstable analysis considering only those individuals that were mapped during collection. These individuals make up the clones analyzed in the previous line of the table.

type consisted of three bands, one corresponding to the fast band, another to the slow band, and a third intermediate band equidistant between the other two. This pattern suggests two alleles segregating at a single locus with the enzyme having a dimeric structure and random association of subunits, as is commonly observed for PHI (Wilkins and Mathers, 1974 and references therein). Following standard practice, it can be assumed that the phenotypic classes reflect the genotypes of the animals, which are designated f/f and s/s for the homozygotes and f/s for the heterozygotes. No rare alleles were observed. The gene frequencies and genotype distributions, together with analysis for goodness of fit to Hardy-Weinberg expectations, appear in Table I.

Woods Hole

The "fast" allele is the most frequent in Woods Hole, as it is in the other locations (Table I). There is a slight excess of heterozygotes over Hardy-Weinberg expectations, but the excess is not statistically significant.

Cape Cod Canal

If the data from all individuals collected at the canal are pooled and analyzed for departure from Hardy-Weinberg expectations, there is a striking deficiency of heterozygotes (Table I). Such a deficiency is commonly produced when separate populations are pooled and analyzed as one (the Wahlund effect). That an analogous phenomenon may be operative here is supported by the observation that this departure is produced as the result of a large aggregation of s/s individuals found on a single rock (station B-2). To emphasize this point, when the data from station B-2 are omitted from the analysis, the population is virtually in Hardy-Weinberg equilibrium (Table I). While detailed mapping was not possible here, station B-2 at the canal contained the only substantial aggregration of s/s individuals observed during the entire study of 535 polyps, suggesting monoclonal origin. Not surprisingly, the omission of the station containing most of the s/s individuals (16 out of 17 observed at the canal) causes a marked shift in the apparent gene frequency (Table I).

A further indication of the patchy distribution of genotypes here is offered by analysis for heterogeneity of distribution of genotypes among the stations where animals were collected in groups according to the rock from which they were removed. A rows by columns G-test (Sokal and Rohlf, 1969) reveals significant heterogeneity in genotype distribution among stations, either with station B-2 ($G = 48.84$, 8 d.f., $P < 0.001$) or without station B-2 ($G = 18.74$, 6 d.f., $P < 0.005$). There is also marked heterogeneity of distribution of gene frequencies ($G = 34.32$, 4 d.f., $P < 0.001$). By analogy with Barnstable (see below), this probably indicates that cloning is a prominent feature at the canal. Mapping of the sort carried out at Barnstable will be required to settle the question, but such analysis will require another polymorphic locus if the same degree of confidence in clonal assignments is to be achieved, since there is no observed color polymorphism at this location. The somewhat confusing picture that emerges from the

Cape Cod Canal emphasizes the value of the kind of analysis that was possible at Barnstable.

Barnstable Town Boat Harbor

The microdistribution of genotypes and clonal assignments for the five mapped collecting stations in Barnstable Harbor are shown in Figures 1–5.

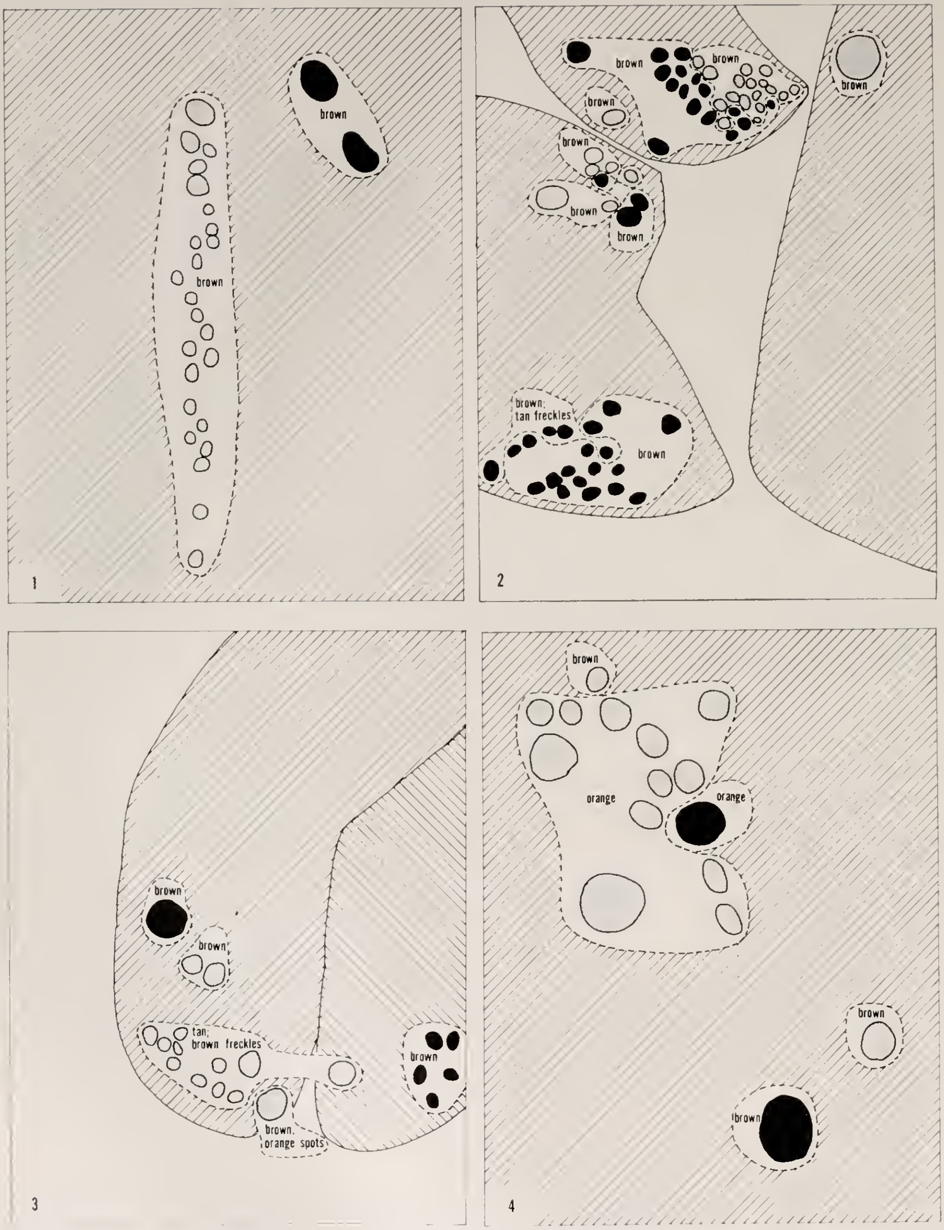
Genotype frequencies of successful larvae do not depart significantly from Hardy-Weinberg expectations (Table I). This can be inferred because the clonal boundaries can be determined and each clone results from a single larva settling from the water column. Furthermore, there is no significant heterogeneity among the five mapped stations in the distribution of genotypes at the time of establishment of successful polyps ($G = 3.14$, 8 d.f., $P > 0.90$). That is, the larvae appear to be successful in establishing new polyps at random with respect to PHI genotype.

When the analysis for goodness of fit to Hardy-Weinberg expectations is carried out using the individual mapped polyps as the genetic unit, there is an extreme departure from expectations (Table I), resulting from an excess of heterozygotes at the expense of both homozygotes. The homozygote deficiency is more pronounced for the s/s individuals. However, the fact that newly established anemones do not depart significantly from expectations and that the clones they produce do depart significantly may not imply that the two genotype distributions differ significantly from each other. In fact, the two genotype distributions (clones *vs.* polyps making up the clones) are homogeneous ($G = 1.31$, 2 d.f., $P < 0.70$). The present suggestion of heterozygote excess is considerably weakened by the homogeneity of the two distributions as they presently stand. Further, analysis of variance for an association of clone size with PHI genotype reveals no significant association ($F_{[2,\ 24]} = 0.316$; $0.50 < P < 0.75$).

However, there is significant heterogeneity among the stations in the distributions of genotypes when individual polyps are considered ($G = 54.47$, 8 d.f., $P < 0.001$), which is precisely what would be expected with random establishment of clones (as indicated by homogeneity of clonal genotype distribution with station) and subsequent differential proliferation of genotypes without significant movement of the progeny. While the collections from the first two Barnstable stations did not involve mapping or exhaustive collection of polyps, pooling of all animals collected in Barnstable, both those mapped and those simply collected by station, only confirms the general picture of heterozygote excess (Table I) and heterogeneity of distribution of genotypes ($G = 70.09$, 12 d.f., $P < 0.001$).

Discussion

The tacit assumption so far has been that the individual polyp rather than the clone is the unit upon which selection acts. It is clear that there is cooperation among clonemates in some aggregating anemones, and that the clone is, in fact, the ecological individual. For example, *Anthopleura elegantissima* creates boundaries between clones that are kept free of nonclonemates by aggressive behavior, presumably to reduce competition between clones. Further, if anemones are mixed in the laboratory, they will segregate into clone-specific groups, and there is evidence of cooperative feeding in the wild (Francis, 1973a, b). There is no


brown
brown
1
brown
brown
brown
brown
brown
brown
brown
brown
brown;
tan freckles
brown
2
brown
brown
tan;
brown freckles
brown
brown,
orange spots
3
brown
orange
orange
brown
brown
4

FIGURES 1–4. Distributions of clones and genotypes at collecting stations in Barnstable Town Boat Harbor. Shading indicates PHI genotype, filled animals being f/f, stippled f/s, and open s/s. Clonal boundaries are indicated by dashed lines, and the color of each clone is indicated within the clonal boundary.

present evidence for this kind of behavior in *Metridium*. Clones frequently sit side-by-side in the field without any evidence of separation, and it is not uncommon to see members of one clone that have moved short distances into the middle of an adjacent clone (Figure 2). Further, there is no apparent preference for clonemates to stay especially close to one another (Figures 1, 4, and 5), and there is little opportunity for cooperation in feeding in an animal that is apparently primarily a filter feeder (Stephenson, 1935). Casual observations in aquaria in the laboratory tend to confirm these arguments. So it seems most likely that a newly produced polyp is independent of clonemates, and that the environment acts independently upon each member of a clone.

That is not to say, however, that asexual reproduction is not an important adaptive strategy. Williams (1975) has likened ameiotically-produced offspring to multiple copies of the same lottery ticket and argued that under certain conditions sexual reproduction serves to increase the chances that an individual's offspring will contain a winning combination. This can account for the presence of sexual reproduction in an organism with asexual capacities in the face of the fifty per cent genetic cost of sexual reproduction. Balancing this, however, the presence of an active organism, such as a polyp of *Metridium*, implies that a winning combination has been produced (evidenced by the success of the individual) and that the environment occupied is suited at least to the maintenance of the individual. It may, in this case, be advantageous to reproduce multiple copies of the same "ticket," since it has already proved that it is a winner. It is also possible to argue that large clones increase the chances of a genotype making a contribution

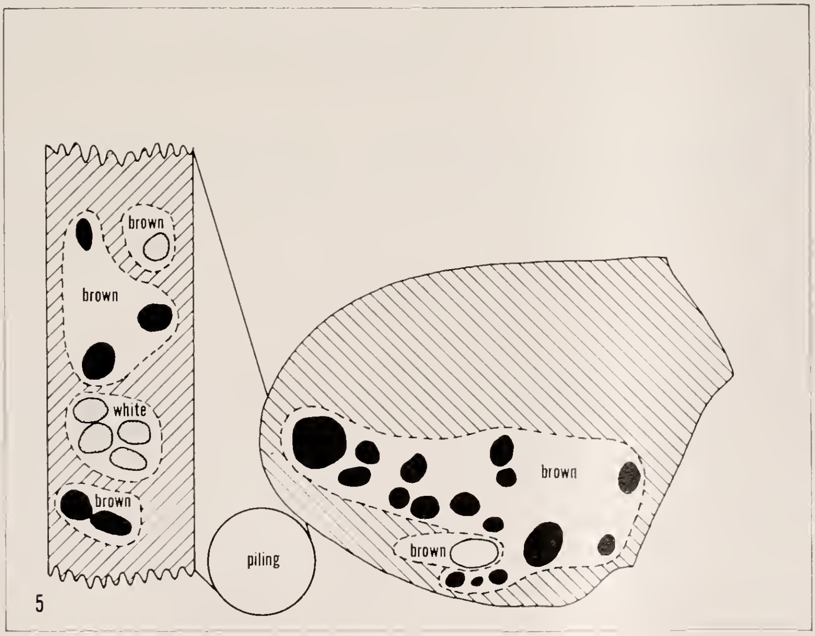


FIGURE 5. Distribution of clones and genotypes at a collecting station in Barnstable Town Boat Harbor. Symbols as in Figures 1–4.

to the next sexual generation. By asexual reproduction it is possible to duplicate the genome without the genetic cost of sexual reproduction.

None of Williams' models of sexual and asexual reproduction seems to fit the *Metridium* case adequately, since they require association between siblings (Williams, 1975, p. 44) to explain the existence of sexual reproduction at all in animals of this type. Such association is extremely unlikely in a planktonically-dispersed organism like *Metridium* (or, indeed, like corals, which, ironically, do not fit his Strawberry-Coral model). Williams' models predict intense selection in organisms of this type, but there is no evidence for this with respect to PHI at least. All gentoypes enjoy some success.

It is tempting to conclude that there is selection for the heterozygotes of PHI at Barnstable. While simple excess of heterozygotes over expectations does not necessarily imply maintenance of the polymorphism by overdominance, there is no significant change in gene frequency during the production of the heterozygote excess (Table I); this would constitute evidence for heterosis as the mechanism maintaining the polymorphism (Lewontin, 1974, pg. 242), assuming that a significant change in gene frequency could be detected with these sample sizes. In fact, the detection of a significant departure from Hardy-Weinberg expectations is a remarkable result, since the departure was detected using a very weak statistical test (see Ward and Sing, 1970 and Lewontin, 1974, for discussion of the lack of power of the χ^2-test to detect departures from Hardy-Weinberg equilibrium with manageable sample sizes). However, as previously stated, the fact that newly established polyps do not deviate from Hardy-Weinberg equilibrium and the polyps that make up the resulting clones do deviate does not necessarily imply that these two groups differ significantly from one another. In fact, as shown above, the genotype distributions of these two groups do not differ significantly from each other. The conclusion of heterozygote superiority must remain speculative.

There is, however, other evidence that PHI genotype may contribute to the success of a polyp at asexual reproduction at Barnstable. There may be a tendency for s/s polyps to produce small clones, since the s/s clones are constituted of one (Figure 5) and three (Figure 2) polyps each. This conclusion would be strengthened by a larger sample size, since only two s/s clones were discovered at Barnstable. Further, the occurrence of the large s/s aggregation at Cape Cod Canal raises the question of whether this result is due to sampling or to locally different selective regimes.

While the lack of mapping and exhaustive collections at Cape Cod Canal and Woods Hole may cloud the issue, there may also be evidence for local differentiation of the three populations. G-test statistics reveal marked heterogeneity of genotype distribution among the three locations, regardless of whether station B-2 from Cape Cod Canal is included in the analysis or not. Unfortunately, without detailed clonal analysis, it is impossible to tell whether the heterogeneity is due to the sampling of several animals from the same clones at a location (meaning that samples at a given location may not be truly independent) or to real differences in gene frequency.

Although this study seems to show tentative evidence that PHI contributes to fitness during asexual reproduction, it is not clear how selection might be operating.

Is the polymorphism maintained by heterosis, or is there a linkage effect that reduces the apparent heterosis to a marker effect for some other locus that is heterotic? Is there some functional disadvantage to the s/s genotype? If so, what balances it? Is balancing selection involved at all? These questions must remain unanswered until data can be obtained on the catalytic properties of the alternative alleles and the heterozygote mixture. Several enzymes show optimum substrate binding only at temperatures experienced by the organism (Somero, 1969; Somero and Hochachka, 1971). It could be that heterozygosity allows optimum binding of substrate over a broader range of temperatures than is possible with only a single enzyme species. Temperature certainly is variable for *Metridium* in the Woods Hole region, annually ranging from 1.8° C to 21.8° C (Sassaman and Mangum, 1970), an important consideration for a sessile animal that cannot escape the variability. But this must remain speculative until data can determine whether it is realistic. Only by accumulating such mechanistic information can the mode of action of selection on this and other enzyme polymorphisms be understood. The investigation of the kinetic properties of PHI variants in *Metridium* will be the subject of continuing investigation in this laboratory.

It is clear, nonetheless, that studies of sessile, asexually reproducing organisms provide powerful tools for the examination of the dynamics of genetic change in natural populations. Not only is it possible to document the distribution of genotypes at some point in the past by inferences about the genotypes of the founders of clones, but it is also possible to measure the success of a given genotype in occupying ecological space by the production of large clones. The resolution possible in this kind of study will be improved with the addition of other polymorphic loci.

I thank J. F. Grassle, J. P. Grassle, C. Sassaman, and J. M. Shick whose comments have substantially improved the manuscript. L. Cole and S. Garner-Price provided valuable field assistance. Experimental work was completed while I was a Postdoctoral Scholar at the Woods Hole Oceanographic Institution. Contribution Number 3716 from the Woods Hole Oceanographic Institution.

SUMMARY

1. *Metridium senile* was studied for phosphohexose-isomerase variation at three locations on Cape Cod, Massachusetts: Woods Hole, Cape Cod Canal, and Barnstable Town Boat Harbor.

2. All three locations exhibited significant polymorphism for PHI.

3. Mapping of individual polyps was performed at Barnstable to analyze spatial distributions of clones and genotypes.

4. In Barnstable, PHI does not depart significantly from Hardy-Weinberg expectations at the time of establishment of new polyps, and establishment of larvae is spatially random with respect to PHI genotype.

5. Asexual reproduction was used as a measure of the relative success of different PHI genotypes. There are indications that not all genotypes are equally likely to produce large clones.

6. There is significant heterogeneity among the three locations with respect to PHI genotype frequencies, suggesting that there may be geographical differentiation of the populations.

7. Sessile, asexual organisms provide powerful tools for examining the dynamic aspects of genetic structure in natural populations.

LITERATURE CITED

Boyer, J. F., 1974. Clinal and size-dependent variation in the LAP locus in *Mytilus edulis*. *Biol. Bull.*, **147**: 535-549.

Campbell, R. D., 1974. Cnidaria. Pages 133–199 in A. C. Giese and J. S. Pearse, Eds., *Reproduction of marine invertebrates, Volume I*. Academic Press, New York.

Costello, D. P., M. E. Davidson, A. Eggers, M. H. Fox, and C. Henley, 1957. *Methods for obtaining and handling marine eggs and embryos*. Marine Biological Laboratory, Woods Hole, 247 pp.

Dobzhansky, T., 1970. *Genetics of the evolutionary process*. Columbia University Press, New York, 505 pp.

Francis, L., 1973a. Clone specific segregation in the sea anemone *Anthopleura elegantissima*. *Biol. Bull.*, **144**: 64–72.

Francis, L., 1973b. Intraspecific aggression and its effect on the distribution of *Anthopleura elegantissima* and some related sea anemones. *Biol. Bull.*, **144**: 73-92.

Fox, D. L., and C. F. A. Pantin, 1941. The colours of the plumose anemone, *Metridium senile* (L.). *Phil. Trans. Roy. Soc. London Ser. B*, **230**: 415–450.

Fox, D. L., G. F. Crozier, and V. E. Smith, 1967. Carotenoid fractionation in the plumose anemone *Metridium*. *Comp. Biochem. Physiol.*, **22**: 177–188.

Gemmill, J. F., 1920. The development of the sea-anemones *Metridium dianthus* (Ellis) and *Adamsia palliata* (Bohad). *Phil. Trans. Roy. Soc. London Ser. B*, **209**: 351-375.

Hahn, C. W., 1905. Dimorphism and regeneration in *Metridium*. *J. Exp. Zool.*, **2**: 225–235.

Koehn, R. K., and J. B. Mitton, 1972. Population genetics of marine pelecypods. I. Ecological heterogeneity and evolutionary strategy at an enzyme locus. *Amer. Natur.*, **106**: 47–56.

Koehn, R. K., and F. J. Turano, and J. B. Mitton, 1973. Population genetics of marine pelecypods. II. Genetic differences in microhabitats of *Modiolus demissus*. *Evolution*, **27**: 100-105.

Lewontin, R. C., 1974. *The genetic basis of evolutionary change*. Columbia University Press, New York, 346 pp.

Sassaman, C., and C. P. Mangum, 1970. Patterns of temperature adaptation in North American Atlantic coastal actinians. *Mar. Biol.*, **7**: 123–130.

Selander, R. K., W. G. Hunt, and S. Y. Yang, 1969. Protein polymorphism and genic heterozygosity in two European subspecies of the house mouse. *Evolution*, **23**: 379–390.

Sokal, R. R., and F. J. Rohlf, 1969. *Biometry*. W. H. Freeman and Co., San Francisco, 776 pp.

Somero, G. N., 1969. Enzymic mechanisms of temperature compensation: immediate and evolutionary effects of temperature on enzymes of aquatic poikilotherms. *Amer. Natur.*, **103**: 517-530.

Somero, G. N., and P. W. Hochachka, 1971. Biochemical adaptation to the environment. *Amer. Zool.*, **11**: 150-167.

Stephenson, T. A., 1935. *The British sea anemones, Vol. II*. The Ray Society, London, 426 pp.

Torrey, H. B., 1902. Papers from the Harriman Alaska expedition. XXX. Anemones, with a discussion of variation in *Metridium*. *Proc. Wash. Acad. Sci.*, **4**: 373-410.

Tracey, M. L., N. F. Bellet, and C. D. Gravem, 1975. Excess allozyme homozygosity and breeding structure in the mussel *Mytilus californianus*. *Mar. Biol.*, **32**: 303–311.

Ward, R. H., and C. F. Sing, 1970. A consideration of the power of the χ^2 test to detect inbreeding effects in natural populations. *Amer. Natur.* **104**: 355–365.

Wilkins, N. P., and N. F. Mathers, 1974. Phenotypes of phosphoglucose isomerase in some bivalve molluscs. *Comp. Biochem. Physiol.*, **48B**: 599–611.

Williams, G. C., 1975. *Sex and evolution*. Princeton University Press, Princeton, 200 pp.

Reference: *Biol. Bull.* **151**: 489–517. (December, 1976)

OBSERVATIONS ON THE FEEDING MECHANISM, DIET AND DIGESTIVE PHYSIOLOGY OF *HISTRIOBDELLA HOMARI* VAN BENEDEN 1858: AN ABERRANT POLYCHAETE SYMBIOTIC WITH NORTH AMERICAN AND EUROPEAN LOBSTERS

J. B. JENNINGS AND S. R. GELDER

Department of Pure and Applied Zoology, University of Leeds, Leeds, England, U.K.

Although the Polychaeta are predominantly free-living annelids, the class includes a number of symbiotic species which live in partnerships of various degrees of intimacy and dependency with host organisms from many different phyla (Clark, 1956; Fauvel, 1959). Relatively little is known of the precise nature of these associations; they are probably based on shelter and nutritional factors, but most of the polychaetes involved do not show the adaptive structural modifications which might be expected and which are evident in other symbiotic annelids such as the Myzostomaria and in symbiotic flatworms such as the Monogenea, Digenea and Cestoda. In these four taxa, however, symbiosis is the only life style; the structural modifications are accompanied, in the flatworms at least, by physiological adaptations which demonstrate the firm nutritional basis of the relationship with the host, and the symbiotes are clearly recognizable as specialized parasites. In contrast, the symbiotic life style is of only sporadic occurrence in the Polychaeta, and in this respect the symbiotic polychaetes are more comparable to those turbellarian flatworms which have adopted symbiotic habits. These too, when compared with their more abundant free-living relatives, show few changes in structure (Hyman, 1951) or, indeed, in diet, feeding mechanisms and digestive physiology (Jennings, 1971), but some species do show differences in food reserves and reproductive strategies which can be related to their life style (Jennings, 1973; Calow and Jennings, 1974). These species are among the most highly adapted of the symbiotic Turbellaria and illustrate possible stages in the evolution of the obligate entoparasites which constitute the wholly symbiotic classes of their phylum.

It is not known whether there is a comparable situation, as regards general nutritional physiology, in symbiotic polychaetes. Of these, the most highly modified morphologically are the members of the family Histriobdellidae Vaillant 1890, all of which live symbiotically in the branchial chamber or on the ventral body surface of crustacean hosts. The histriobdellids are small annelids, rarely exceeding 2 mm in length; they have only nine post-cephalic segments of which the last is bifurcated to form a pair of locomotor-cum-adhesive organs, and segmental appendages are much reduced or absent. Despite these modifications the Histriobdellidae show undoubted affinities with the Eunicida (Mesnil and Caullery, 1922; Remane, 1932; Hermans, 1969), and their position in that order has been confirmed from a study of their nervous system (Gelder and Jennings, 1975).

The family consists of the two genera *Histriobdella* and *Stratiodrilus,* the former with one species *H. homari* van Beneden 1858 symbiotic with marine lobsters in northern European and northeastern American waters (van Beneden, 1853; 1858; Uzmann, 1967) and the latter with four species symbiotic with freshwater Decapoda in Tasmania, Australia, Uruguay, Madagascar, Chile, Argentina and Patagonia, and one with a marine isopod in South Africa (Haswell, 1900; 1913; Cordero, 1927; Harrison, 1928; Lang, 1950; Roubaud, 1962; Führ, 1971).

The unusual features of the Histriobdellidae suggest that they may represent one climax in the evolution of symbiotic habits within the Polychaeta, comparable perhaps to some turbellarian symbioses such as those involving temnocephalid rhabdocoels and decapod crustaceans or umagillid rhabdocoels and echinoids (Jennings, 1971). The nature of the histriobdellid-crustacean relationship, however, has not been defined; the histriobdellids are usually described as parasites (*e.g.*, Mesnil and Caullery, 1922; Fauvel, 1959; Dales, 1967), but no supporting evidence has been given. Virtually nothing is known of their diets or feeding mechanisms; the proboscis and jaws are much modified, but judging from the descriptions by Foettinger (1884), Shearer (1910), Haswell (1913), Mesnil and Caullery (1922) and Roubaud (1962), they are clearly derived from the basic eunicid pattern described by Fauvel (1959) and Dales (1962), suggesting a scraping or browsing type of feeding. The life history is simple, with the females attaching their eggs to the ventral surface and egg masses of the host and the young hatching as miniature adults (van Beneden, 1858; Haswell, 1913). Transference between hosts is probably accomplished by direct migration, as demonstrated by Simon (1967; 1968) for *H. homari* under laboratory conditions.

H. homari is a common symbiote of European and North American lobsters (van Beneden, 1858; Uzmann, 1967) and, if parasitic, may be of some economic importance. It shows all the unusual features of its family and it has, therefore, been selected for study as an example, albeit a somewhat extreme one, of symbiosis in the Polychaeta. Since most symbioses have a nutritional basis, the investigation has been concerned with the structure of the alimentary canal, the diet, feeding mechanism and general digestive physiology, with the aim of establishing the status of *H. homari vis-à-vis* its host and facilitating comparisons of polychaete symbioses with those found in the Platyhelminthes and other phyla.

MATERIALS AND METHODS

Histriobdella homari was collected from the gills and epipodites of lobsters, *Homarus americanus,* caught at Nahant and Woods Hole, Massachusetts, and *H. vulgaris* caught at Whitby, England.

The structure of the buccal cavity, proboscis, alimentary canal and their associated glands, and the nature and role of the various substances secreted by the latter, were studied by histological and histochemical methods. For histological and nonenzymic histochemical studies, specimens were fixed in marine Bouin's fluid, Flemming's fixative, 10% neutral formalin or 95% ethanol. Whole mounts and serial sections cut at 5 μm after dehydration in graded ethanols and impregnation in polyester wax (melting point, 39° C) were stained with Ehrlich's haematoxylin and eosin, Gram's stain or Mallory's triple stain, or by the periodic acid-Schiff reaction for carbohydrates and acid mucopolysaccharides, Steedman's Alcian blue method for acid mucopolysaccharides, Feulgen's reaction for DNA, the

Sudan IV and Oil red 0 methods for lipids and Best's carmine method for glycogen.

For histochemical studies of digestive enzymes fixation was in 10% formalin, buffered with phosphate to pH 7.0, at 1° C. Whole mounts, and serial sections cut at 10 μm after dehydration in graded acetones and impregnation in paraffin wax (melting point, 45° C), were examined by the indoxyl acetate method for non-specific esterases (Holt, 1958), the L-leucyl β-naphthylamide hydrochloride method for arylamidases (Burstone and Folk, 1956), the naphthyl AS-BI phosphate methods for acid and alkaline phosphatases (Burstone, 1958) and the post-coupling 6 bromo-2-naphthyl-β-D-glucopyruronoside (glucuronide) method for β-glucuronidase (Pearse, 1972). The esterases demonstrated by Holt's method were characterized further using specific inhibitors and activators and following procedures and interpretations given by Pearse (1972) and Hassall and Jennings (1975).

Negative controls for the enzyme studies consisted of heat inactivated specimens and sections (held at 90° C for two minutes before incubation) and the omission of specific substrates from incubation media; positive controls consisted of simultaneous processing of appropriate mammalian and molluscan tissues.

The structure of the proboscis was also studied by digesting the head at room temperature in 0.5% pepsin in 0.2% hydrochloric acid; the various components of the jaw apparatus could then be separated by gentle pressure on a coverslip placed over the preparation. The operation of the proboscis was investigated by direct observation of living *H. homari in situ* on excised host gill filaments and epipodite setae. This was supplemented by photographing the proboscis in movement using "Ilford Mark V" 16 mm negative film at sixty-four frames per second in a Paillard Bolex H 16 cine camera with subsequent frame-by-frame analysis in an L. W. 900 B Motion Analyzer (L. W. Photo Inc., Van Nuys, California).

The nature of the food and the site and sequence of its digestion were studied partly by direct observation of living *H. homari,* as described above, but principally from examination of the gut contents of individuals fixed immediately after removal from the host and then subjected to one or other of the various histological and histochemical routines. The host's gill filaments, epipodites, epipodite setae and the lining of its branchial chamber were similarly examined, to ascertain the source and original condition of the gut contents and to allow differentiation between enzymes ingested as components of the food and those secreted by *H. homari.* pH conditions attending digestion were measured by intra-vital staining with 0.01% sea water solutions of bromo-cresol green (pH range 3.8–5.4), bromo-cresol purple (5.2–6.8), bromo-thymol blue (6.0–7.6) and phenol red (6.8–8.4).

The observations on the cellular structure of the proboscis, stomach and intestine, and on the nature of the food, were supplemented by ultrastructural studies. Fixation, dehydration, impregnation in epon and the preparation of sections for examination with the light and electron microscopes followed the procedures described by Jeon (1965), Parke and Manton (1967) and Jennings (1969).

Observations and Results

Structure of the alimentary canal

The alimentary canal in *Histriobdella homari* (Figs. 1A and 1B) consists of a mouth, small buccal cavity, oesophagus, proventriculus, stomach, intestine and

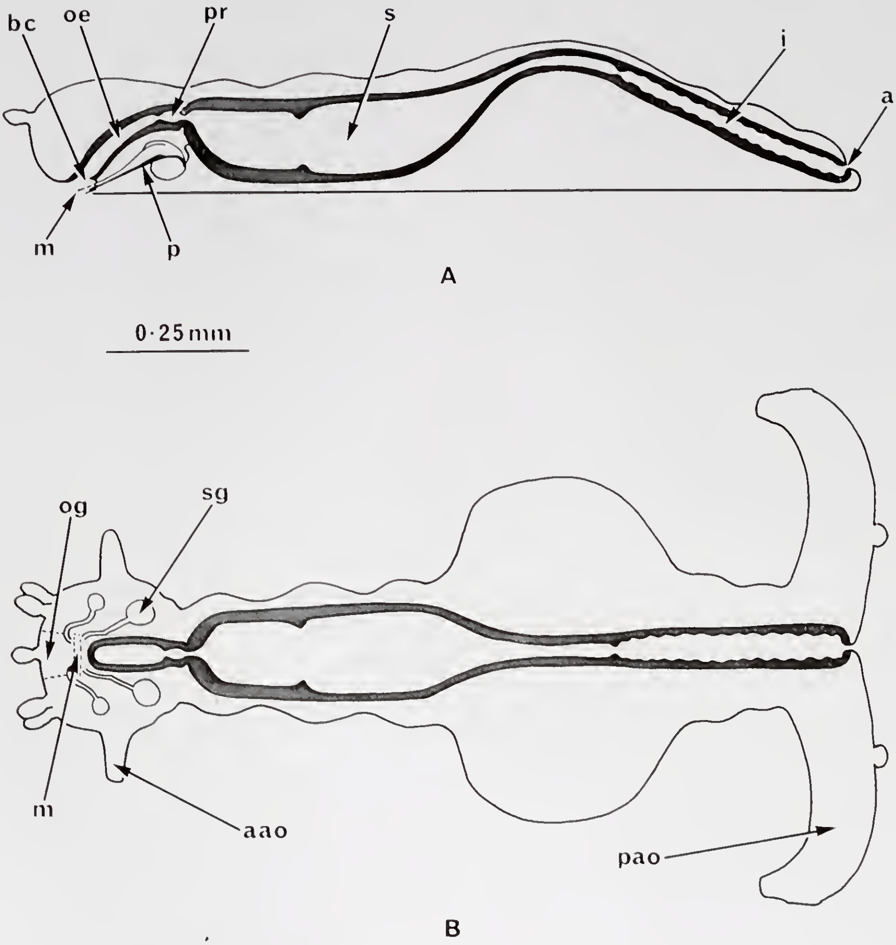


Figure 1. *Histriobdella homari.* Schematic longitudinal sections (A, near-sagittal and B, horizontal) through a mature male to show the alimentary canal and proboscis. Abbreviations are: a, anus, aao, anterior adhesive organ; bc, buccal cavity; i, intestine; m, mouth; oe, oesophagus; og, oral groove; p, proboscis; pao, posterior adhesive organ; pr, proventriculus; s, stomach; sg, salivary gland (only two of the nine pairs are shown). The scale bar indicates the overall dimensions; the gut wall is shown thicker than in life.

anus. There is a cluster of unicellular salivary glands on each side of the oesophagus and the modified nonprotrusible proboscis lies in the mid-ventral region of the head below the oesophagus with its anterior portion protruding into the buccal cavity.

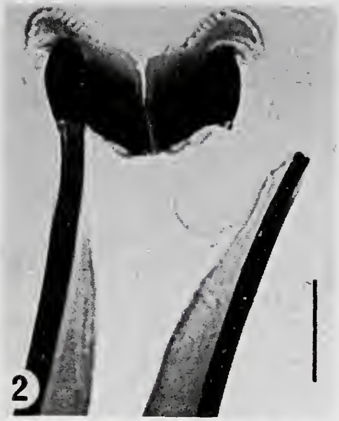
The mouth, buccal cavity and proboscis. A shallow oral groove, 30–40 μm wide, originates at the anterior margin of the head and runs posteriorly on the ventral surface to the transverse slit-shaped mouth (Fig. 1B). The mouth is 40–45 μm by 8–10 μm at rest but is capable of considerable distension during feeding. It opens vertically into a small ovoid buccal cavity which in turn leads dorsally into the oesophagus and receives posteriorly the tips of the maxillae and mandibles of the proboscis (Fig. 1A). The oral groove, mouth and buccal cavity are lined by an unciliated epithelium covered by a thin flexible cuticle, both of which are continuous with those covering the general body surface.

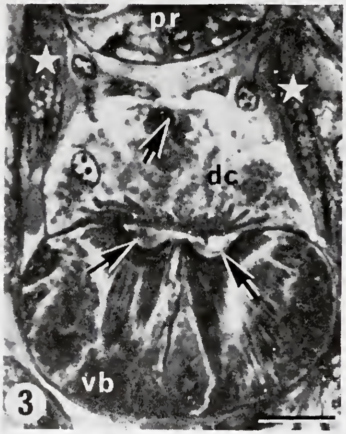
The proboscis (Figs. 1 and 18) is one of the more obvious features of *H. homari,* being easily visible in the light-colored living animal. Previous descriptions have been almost entirely limited to accounts of the sclerotized parts and have varied in detail and accuracy; the most comprehensive account is that by Mesnil and Caullery (1922), but even these authors omit details of the associated musculature and do not attempt the functional interpretation needed for an understanding of the role of the proboscis in the procuring and ingestion of food. To do this it is necessary to give here a full description of the entire proboscis apparatus as observed in the present study. Previous accounts have used different terms to describe the same sclerotized parts, leading to considerable confusion; but, in view of the eunicid nature of the histriobdellids, the basic terminology used by Dales (1962) to describe the proboscis and jaws of typical eunicids will be adopted whenever obvious homologies permit. Synonyms from Haswell (1900, 1913), Shearer (1910) and Mesnil and Caullery (1922) are given in parentheses.

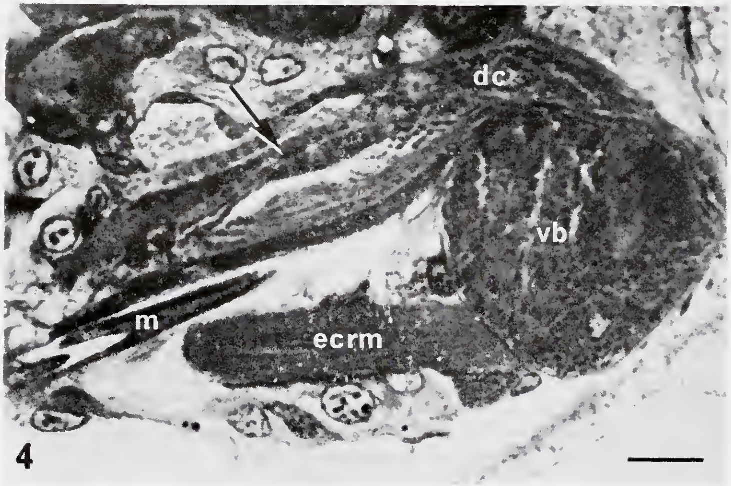
The proboscis in *H. homari* (Figs. 2–14) is 100–120 μm long and 40–45 μm wide. Its hard components are composed of a tough dark sclerotized material and consist of two parallel ventral mandibles, a single flexible median dorsal rod ("fulcrum"), paired series of denticulate maxillae ("rami" and "ramules") and a single transverse carrier ("bridle") which slides backward and forward upon the mandibles. Muscular components include a posterior muscular organ, subdivided into a ventral subspherical bulb and a dorsal pyriform structure which has anterior muscular extensions, lateral paired retractor muscles, posterior dorsoventral muscles which anchor the posterior muscular organ to the oesophagus and walls of the head, and anterior muscles which link the maxillae and mandibles to the two major longitudinal muscle blocks of the general body musculature.

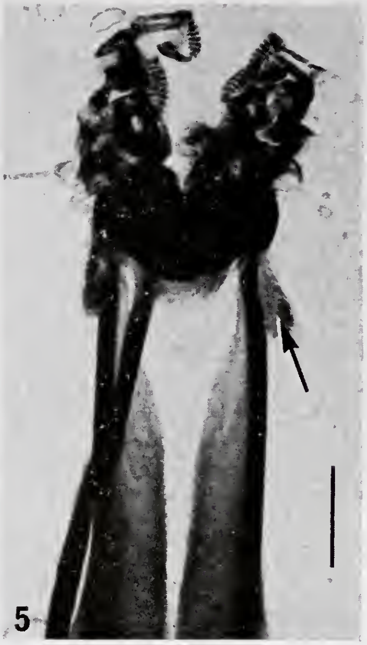
The mandibles (Figs. 2 and 12) are rigid fixed structures 95–110 μm long which lie ventrally behind the buccal cavity and below the oesophagus. Each is expanded anteriorly into an outwardly directed hook-shaped portion and an inner rhomboidal plate which touches, but does not fuse with, its fellow on the other mandible. The anterior margins of the plates and hooks are serrated and protrude through the buccal epithelium into the rear of the buccal cavity; the posterior margins are thickened and heavily sclerotized. Each plate is perforated near its posterior margin by an aperture 1–1.5 μm in diameter; the two apertures are linked by a solid non-elastic strand of hyaline tissue which runs between them across the ventral surfaces of the plates, and this holds the plates together.

Behind these expansions the mandibles become progressively more j-shaped in cross section (Figs. 12C and 12D). They develop a vertical plate on their outer margins 3–4 μm high, whose upper edge is thickened over most of its length into a rod 1.5–2 μm in diameter, and also expand laterally on their inner surfaces into blade-like processes which are shallowly curved in cross-section and almost meet in the mid-line. Posteriorly the mandibles are attached to the flattened dorsal surface of the ventral bulb of the posterior muscular organ, retaining their j-shape as they pass over the anterior margin of the bulb but then quickly losing it as their vertical plates taper away (Fig. 3). The horizontal blades become less

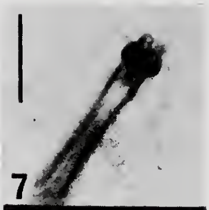

2

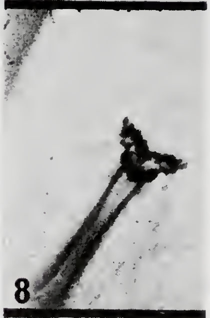

3

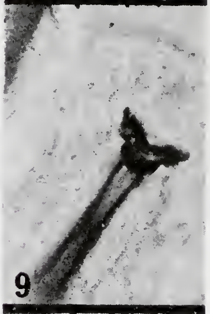

4

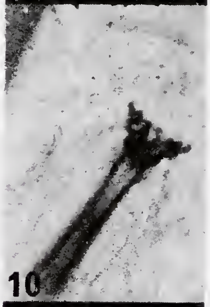

5

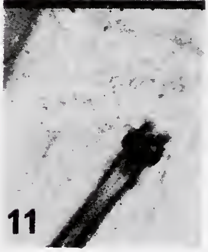
6


7


8


9


10


11

curved in cross-section and less sclerotized as they pass over the surface of the bulb and curve ventrally around its posterior margin to terminate about one third of the way down the posterior surface (Fig. 14).

The median dorsal rod of the proboscis (Figs. 3, 5, 12, 13 and 14) lies in the mid-line above the mandibles and is inclined from these posteriorly at an angle of approximately 10°. It is 75–80 μm long, 1.5–2 μm in diameter, with a slight dorsal bowing in its posterior half and with its anterior tip slightly bifurcated. The posterior half is embedded within a single narrow elongated cell, 35–40 μm long, which surrounds the rod as a close-fitting sheath and is itself embedded in the upper part of the dorsal component of the posterior muscular organ 12–15 μm above the posterior regions of the mandibles (Figs. 3 and 14).

The posterior end of the rod is surrounded by many folded membranes which anchor it securely within the cell; ultrastructurally these have the appearance of degenerate rough endoplasmic reticulum and thus may well have been concerned with the initial formation of the rod. The cell wall around this region is considerably thickened and consists of a homogeneous matrix enclosed within inner and outer limiting membranes; this thickened area prevents any appreciable backward movement of the rod during operation of the proboscis.

The four pairs of maxillae (Figs 5 and 13) lie above the anterior portions of the mandibles. Each maxilla consists of a series of articulated sclerotized components embedded in the epithelium and musculature of the postero-lateral walls of

---

FIGURE 2. *H. homari.* The anterior portions of the mandibles photographed from the dorsal aspect after digestion of the proboscis musculature and removal of other sclerotized components. The right mandible fractured during preparation of the specimen and the longitudinal component is displaced to the right. Scale is 10 μm.

FIGURE 3. Transverse section through the posterior muscular organ of the proboscis. The arrows point to the mandibles and dorsal rod which are seen in cross-section and the stars indicate the dorso-ventral muscles which anchor the proboscis posteriorly; dc, dorsal component; vb, ventral bulb; pr, ventral wall of the proventriculus. The section is of epon-embedded material and is stained with Azur II; scale is 10 μm.

FIGURE 4. Nearly sagittal longitudinal section through the proboscis slightly to the left of the mid-line. The arrow points to the left dorsal rod tensor muscle where this passes over the flexor muscles of the first maxilla; below these can be seen portions of the dorsal rod flexor muscles and the upper margin of the left mandible; dc, dorsal component of the posterior muscular organ; ecrm, left external carrier retractor muscle; m, portion of left mandible; vb, ventral bulb of the posterior muscular organ. The section is of epon-embedded material and is stained with Azur II; scale is 10 μm.

FIGURE 5. The maxillae, carrier and anterior portions of the mandibles and dorsal rod from the dorsal aspect after digestion of the proboscis musculature. The dorsal rod is displaced to the left. The arrow indicates the right-hand expanded wing of the carrier; scale is 10 μm.

FIGURE 6. The carrier from the dorsal aspect after separation from the rest of the proboscis apparatus; scale is 10 μm.

FIGURES 7–11. Five consecutive frames from a filmed record of the proboscis in action, ventral aspect, showing an uninterrupted cycle of proboscis movements without pause for independent operation of the first maxillae. The frames show the carrier and maxillae in the course of protraction (Fig. 7), the point of maximum extension of the maxillae (Fig. 8), stages in retraction of the carrier and maxillae during the effective feeding stroke (Figs. 9 and 10), and the point of maximum retraction (Fig. 11). Photographed at 64 frames per second, giving a time of approximately 75 milliseconds for the complete uninterrupted cycle. Scale is 50 μm.

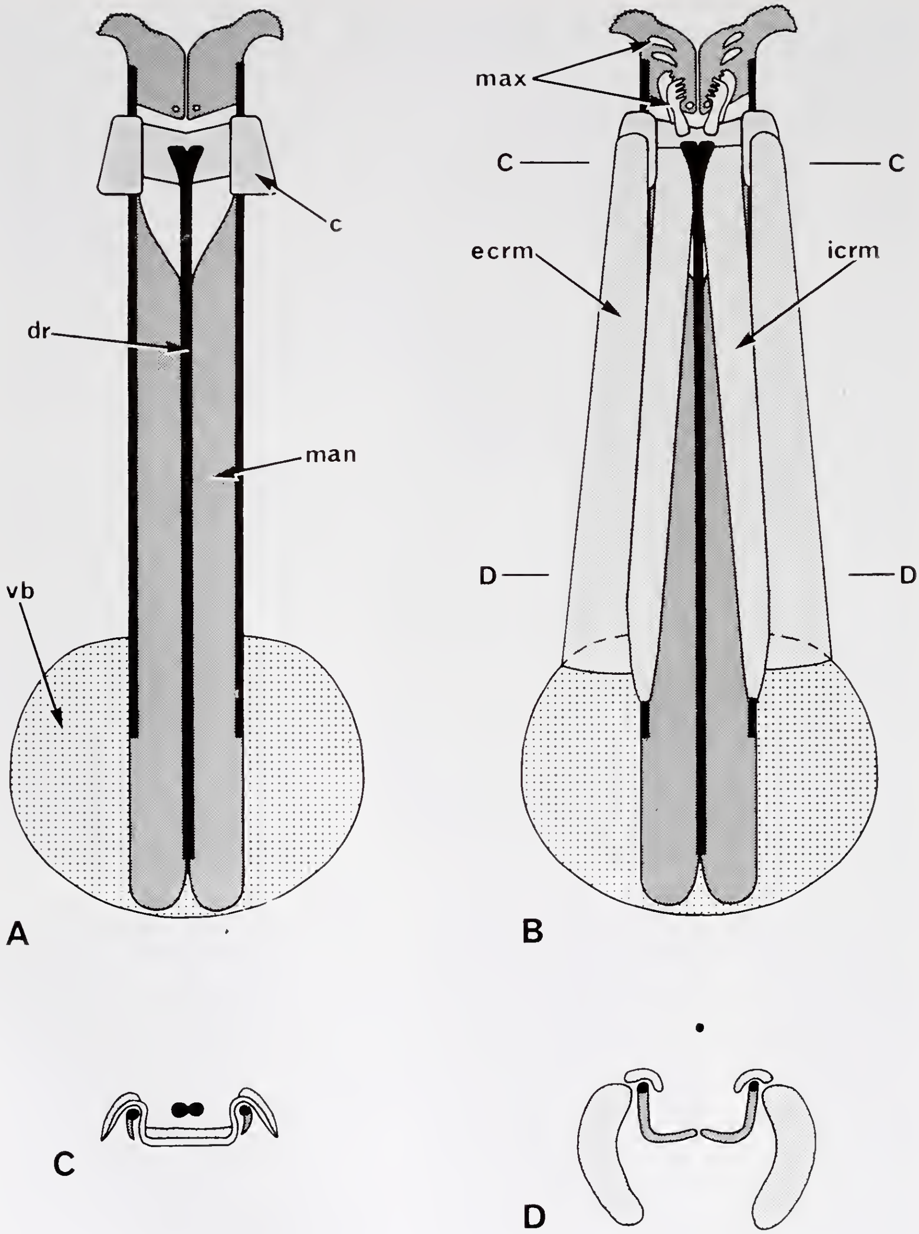


FIGURE 12. *H. homari.* Schematic diagrams of the proboscis to show the arrangement and relationships of the parts, excluding the dorsal component of the posterior muscular organ and the muscles derived from it. A) The mandibles, carrier, dorsal rod and ventral bulb from the dorsal aspect. B) As A, but with the addition of the carrier retractor muscles and the maxillae. C) Transverse section through the proboscis in the plane C-C. D) Transverse section in the plane D-D. Abbreviations are: c, carrier; dr, dorsal rod; ecrm, external carrier retractor muscle; icrm, internal carrier retractor muscle; man, mandible; max, maxillae; vb, ventral bulb of the posterior muscular organ.

the buccal cavity. The number, size and shape of the component pieces varies in the different maxillae, but the distal one is always the largest; it protrudes from the buccal epithelium into the buccal cavity and bears on its exposed surface either teeth or series of ridges.

The innermost pair of maxillae, which will be called the first maxillae, are 12–15 μm long and 4–5 μm wide. They each consist of a small cuboidal basal element, which articulates proximally with the tip of its respective branch of the bifurcated anterior end of the dorsal rod, and a much larger columnar component. The latter articulates at its base with the distal end of the basal element, and most of its length protrudes from the buccal epithelium. The distal third of the inner surface of the exposed portion of the column, *i.e.,* the surface facing the other first maxilla, is produced into four large spike-like teeth. These toothed components, unlike their homologues on the second, third and fourth maxillae, can be moved independently of the other parts of the maxillary apparatus. They can be swung inward, from a position in which they lie almost parallel to the surface of the buccal epithelium, through an arc of 70–80° (Fig. 13A) almost to meet before returning to their original position.

The second maxillae are 15–20 μm long and lie ventrally to the first pair. Each consists of three components: a basal piece which lies below the corresponding element of the first maxilla of its side and, like that, articulates with the dorsal rod, a small median element and a columnar distal component. The latter is larger than its homologue of the first maxilla but is less exposed, having the greater part of its column embedded in the buccal epithelium (Fig. 13). The exposed surface bears eight transverse ridges which give it a file-like appearance.

The third maxillae are 25–30 μm long and are each composed of six rod-shaped elements. The first three elements are arranged linearly, with the proximal one articulating with the basal region of the columnar distal component of the second maxilla. The remaining three elements form a triangle which lies at an acute angle to the line of the first three, with the distal component forming the hypotenuse (Fig. 13). This distal component is 7–8 μm long and 2–3 μm wide; it is slightly curved along its long axis and its exposed surface bears nine transverse ridges.

The fourth maxillae are 12–14 μm long and are each composed of four roughly rod-shaped elements which are arranged in a similar pattern to those of the third maxillae. The proximal element articulates with the third element of the third maxilla and the distal one bears nine transverse ridges on its exposed surface.

The carrier (Figs. 6 and 12) is a trough-shaped structure 4–5 μm long, 8–9 μm wide and 2–3 μm deep which lies between the mandibles anteriorly. Dorsally it has lateral wing-like expansions 3–4 μm wide and 8–9 μm long which rest on the vertical blades of the mandibles so that the carrier is suspended between these (Fig. 12C). During operation of the proboscis apparatus the carrier slides backward and forward along the mandibles over a distance of 12–15 μm from the anterior end of the vertical blades.

A series of fine inelastic fibers ascend inward from the base and walls of the anterior half of the carrier to the two short limbs of the bifurcated tip of the dorsal rod. These bind the rod to the carrier so that movement of the carrier along the mandibles causes a similar movement of the rod tip, and *vice versa.* The link is not rigid, however, and the carrier and rod tip can move independently of each other over 1–2 μm.

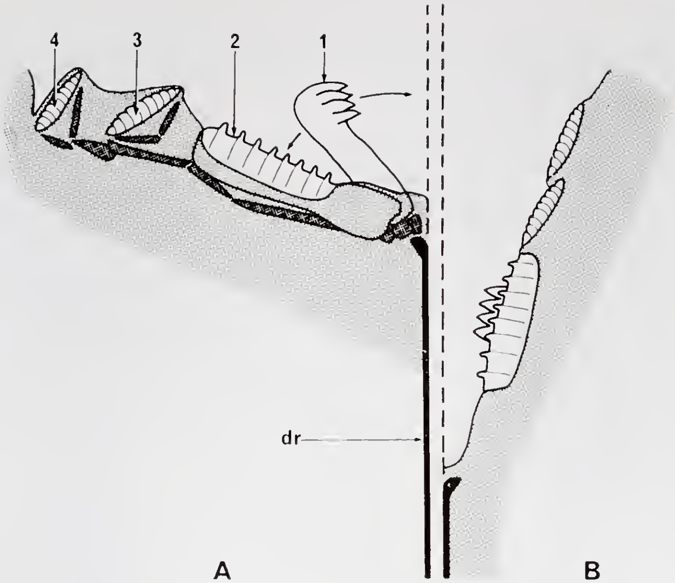


Figure 13. *H. homari.* Schematic diagrams of the maxillae from the ventral aspect showing A) the positions of the right maxillae when fully extended laterally and B) the positions of the left maxillae when partially retracted into the resting position. 1, 2, 3 and 4 indicate the exposed denticulate or ridged surfaces of the distal components of the first, second, third and fourth maxillae; the arrows on each side of the first maxilla indicate its arc of movement; dr indicates the right half of the dorsal rod. The dorsal rod and the embedded components of maxillae 1–4 are shown in black.

Similar fibers ascend outward from the anterior margins of the base and sides of the carrier to the basal components of the first and second maxillae, while others run posteriorly from these structures to the two limbs of the dorsal rod.

The musculature of the proboscis is concerned with protraction and retraction of the maxillae relative to the mandibles during feeding, and with anchoring the entire apparatus within the head. The ventral bulb of the posterior muscular organ (Figs. 3, 4, 12 and 14) has the form of a truncated sphere, 25 μm tall and 36 μm in diameter, and is composed of ten conical muscle cells enclosed within a thin membranous sheath. The cells have large basal nuclei with prominent masses of chromatin and contain peripheral bundles of striated fibers (Figs. 3 and 4), very similar to those of the muscle cells of the general body musculature, and many large mitochondria. The bundles of fibers run the length of the cells and converge in the apical regions beneath the ventral surfaces of the mandibles where these are attached to the bulb; their function appears to be the establishment and maintenance of tension within the bulb during operation of the proboscis apparatus and especially during contraction of the external carrier retractor muscles whose posterior ends are attached to each side of the bulb.

The mitochondria are interspersed between the bundles in the peripheral regions of the cells and are densely packed together in the inner regions where they almost obliterate other organelles.

The dorsal component of the posterior muscular organ (Figs. 3 and 4) is a dome-shaped structure, 12–14 μm tall and 25–30 μm wide, which lies above the ventral bulb with its posterior region extending ventrally over the postero-lateral surfaces of the latter for 5–6 μm (Fig. 14A). It has one median, two lateral and two dorso-lateral anterior extensions which form muscles concerned with the functioning of the dorsal rod in the operation of the proboscis apparatus and the movements of the first maxillae.

The dorsal component consists of nine large muscle cells which, like those of the ventral bulb, contain bundles of striated fibers and many mitochondria. They are, however, oriented at right angles to the ventral bulb cells, so that their long axes are parallel to the mandibles and dorsal rod. The nuclei and most of the

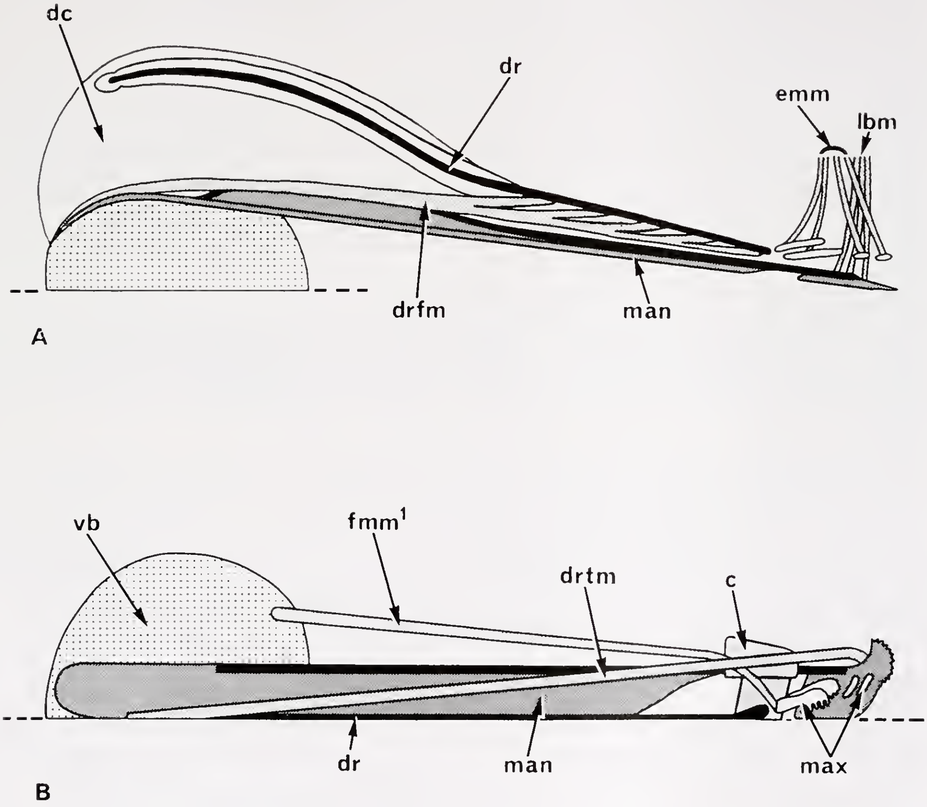


FIGURE 14. *H. homari.* Schematic projections of the dorsal region of the proboscis (left side) to show the distribution of the muscles associated with the dorsal rod and maxillae. A) Sagittal projection of the left half; for clarity the carrier is not represented. B) Dorsal projection; cell bodies of dorsal component not represented. Abbreviations are: c, carrier; dc, dorsal component of the posterior muscular organ; dr, dorsal rod; drfm, dorsal rod flexor muscle; drtm, dorsal rod tensor muscle; emm, extensor muscles of the maxillae; fmm^1, flexor muscle of the first maxilla; lbm, anterior ends of the longitudinal body muscles which are attached to the mandibular hook; man, mandible; max, maxillae; vb, ventral bulb.

mitochondria lie in the broader posterior regions of the cells, which make up the body of the dorsal component; the bundles of striated fibers also originate here and pass forward into the anterior extensions of the cells (Fig. 4).

The nine cells are arranged in two tiers. The upper tier contains only two cells; these lie one on each side of the narrow elongated cell which contains the posterior portion of the dorsal rod. The two cells extend over this inner cell as a thin layer 5–6 μm thick, but the bulk of their bodies lies laterally and ventrally to it. The anterior portion of each cell extends forward and downward, diverging from the midline, and becomes attached to the posterior margin of the mandibular hook of its respective side. Two bundles of striated fibers originate in the regions immediately adjacent to the posterior tip of the dorsal rod and run forward for the full length of the cells to their insertion on the mandibular hooks. The two cells thus form a pair of muscles which help prevent any backward movement of the rod during operation of the proboscis, and are named, therefore, the dorsal rod tensor muscles (Figs. 4 and 14B, drtm).

Five of the seven cells in the lower tier are arranged so that their posterior portions form a compact block, and it is the postero-lateral margins of this which extend downward over the ventral bulb (Fig. 14A). Each cell contains a single bundle of striated fibers which originates in that part of the cell overlapping the ventral bulb. The bundles pass into the anterior extensions of the cells which run forward beneath the dorsal rod and become successively attached to the ventral surface of its anterior half, beginning just beyond the point where the rod arches posteriorly.

The five cells thus form a single functional unit whose contraction supplements the action of the carrier retractor muscles, which will be described later, in causing posterior movement of the rod tip and carrier and concomitant bowing of the dorsal rod. The unit is named, therefore, the dorsal rod flexor muscle (Fig. 14A, drfm).

The remaining two cells of the dorsal component lie antero-laterally to the block of five cells. The anterior extension of each cell, containing a single bundle of striated fibers, runs forward to the first maxilla of its respective side and is attached to the toothed distal component. Contraction of the fibers causes this to swing inward towards its fellow of the other first maxilla; the two cells thus form a pair of muscles responsible for flexure of these structures and are consequently named the flexor muscles of the first maxillae (Fig. 14B, fmm^1).

Two pairs of muscles, the external and internal carrier retractor muscles, run from the ventral bulb of the posterior muscular organ to the carrier (Figs. 4 and 12). The external retractors run along the outer sides of the mandibles; each consists of a single multinucleate cell which is kidney-shaped in cross section over most of its length with the concave surface facing inward (Fig. 12D). The cell is 45–50 μm long and 20–22 μm tall posteriorly where it is attached to the ventral bulb. It tapers anteriorly, while still retaining its characteristic cross-sectional shape, and becomes attached to the dorsal surfaces of the lateral wing-like extensions of the carrier (Figs. 12B and 12C).

The internal carrier retractors are attached posteriorly to the upper part of the vertical blades of the mandibles, where these pass over the anterior margin of the ventral bulb (Fig. 12B). Each consists of a single mononucleate cell which has the same kidney-shaped cross section as the external retractors but they are oriented so that the concave surface lies over the upper thickened edge of the mandibles (Fig. 12D). They run forward and slightly inward from their point of attachment, descending gradually until they become attached to the floor of the carrier (Fig. 12C).

The four cells forming the external and internal carrier retractor muscles resemble the muscle cells of the posterior muscular organ in that they contain bundles of striated fibers and very many large mitochondria, the only significant difference being the multinucleate condition of the external retractors. These each have four large nuclei in their posterior portions but there are no traces of any internal partitions of the cell.

The bundles of fibers in the external retractor muscle cells are attached posteriorly to the internal surface of the cell membrane; the points of attachment lie directly over the attachments of many of the fibers within the ventral bulb cells, and thus the two sets of fibers form an antagonistic system. Contraction of the bulb fibers causes rigidity of the bulb, as described earlier, and this provides a firm basis for the contraction of the external retractor fibers comparable to that provided by the vertical blade of the mandibles for the internal retractors. Contraction of both pairs of retractors pulls the carrier posteriorly along the mandibles and a consequence of this is a similar posterior movement of the maxillae and the anterior tip of the dorsal rod.

The musculature associated with the anterior end of the proboscis is derived from two large longitudinal muscle bands which run the length of the body and are the principal dorsal constituents of the general body musculature. The majority of the bundles of fibers within these muscles terminate in the paired anterior adhesive organs (Fig. 1B) but the remainder descend steeply on each side of the oesophagus toward the mouth. Small bundles of fibers run to the bases of the exposed distal components of the first, second, third and fourth maxillae, while others run to the lateral margins of the mandibular hooks. Contraction of the fibers running to the maxillae causes the distal components to move outward; the fibers therefore constitute extensor muscles of the maxillae (Fig. 14A, emm).

The fibers running to the mandibular hooks do not cause movement of any part of the proboscis apparatus, and they appear simply to be the means of anchoring the longitudinal muscle bands to fixed points within the head, the mandibles being embedded anteriorly in the buccal epithelium and its underlying tissues.

The proboscis apparatus is anchored within the head posteriorly by lateral dorso-ventral muscles (Fig. 3, stars). These run vertically from the ventral wall of the head on each side of the posterior muscular organ and proventriculus, curve inward over the proventriculus and join dorsally in the midline beneath the epidermis. They are thin sheets 2–3 μm thick and 9–10 μm wide which are firmly attached to the sides of the ventral bulb immediately behind the attachments of the external carrier retractor muscles.

A single thin sheet of noncontractile tissue ascends obliquely backward from the mid-posterior surface of the ventral bulb to the anterior wall of the stomach; the function of this connective is not apparent, but it may contribute to the anchoring of the proboscis apparatus.

The elaborate innervation of the proboscis has been described elsewhere (Gelder and Jennings, 1975). It consists basically of a pair of supraproboscidial ganglia linked directly with the brain by two short stout nerves, longitudinal connectives, transverse commissures, and paired nerves which serve all the various muscular components described here.

Operation of the proboscis. When the proboscis apparatus is at rest, the carrier retractor muscles are slightly contracted so that the carrier lies approximately one third of the way along the length of its posterior travel, with its anterior margin 4–5 μm from the anterior ends of the vertical blades of the mandibles. The flexible dorsal rod, which is attached at its anterior tip to the carrier, is therefore under some tension as backward movement of its posterior end is prevented by the thickened posterior wall of the cell enclosing it and the restraining action of the dorsal rod tensor muscles. This tension, however, is not enough to cause any bowing of the rod in excess of the intrinsic structural curvature of its posterior half. The dorsal rod flexor muscle is slightly contracted, and this holds the anterior tip of the rod down against the carrier so that any upward movement of either the rod or carrier is prevented.

The first and second maxillae are drawn slightly posteriorly since they are connected by their basal elements to both the carrier and the tip of the dorsal rod. The basal elements are firmly embedded in the wall of the buccal cavity and the pull upon them is therefore transmitted to the third and fourth maxillae which are similarly embedded. Thus, in the resting position, the four maxillae of each side lie one behind the other in lines which subtend angles of approximately 20° to the mid-line (shown for the left maxillae, ventral aspect, in Fig. 13B). The extensor muscles of the maxillae are relaxed, allowing withdrawal of the maxillae to this position.

The cycle of movements of the proboscidial components, from this resting position, is initiated by further contraction of the carrier retractor muscles. This pulls the carrier back along the mandibles for another 10–12 μm to the posterior limit of its travel. The force acting on the dorsal rod is thus greatly increased as its anterior end moves posteriorly with the carrier; this is accommodated by an increase in the dorsal curvature of its posterior half. The dorsal rod tensor and flexor muscles also contract and continue to exert their restraining actions on both ends of the rod. The maxillae are pulled further posteriorly and the denticulated series of the distal components almost meet their fellows of the opposite side when the carrier retractor muscles are at maximum contraction. This movement of the maxillae is accompanied by further relaxation of the extensor muscles of the maxillae and some stretching of the posterior wall of the buccal cavity.

Forward movement of the carrier to the anterior limit of its travel and simultaneous forward and outward movement of the maxillae are achieved primarily by sudden relaxation of the carrier retractor muscles, which allows the dorsal rod to return to its original shape by a rapid forward movement of its anterior end. The dorsal rod flexor muscle permits this by relaxing to an appropriate degree, and the extensor muscles of the maxillae actively contribute to it by contracting. The extensor muscles contribute mainly to the outward movement of the maxillae and further supplementation comes from release of tension in the stretched wall of the buccal cavity. As the maxillae move outward continued contraction of the extensor muscles causes the third and fourth maxillae to swing through an arc of 120° so that the exposed ridged surfaces of their distal components come to lie almost at right angles to those of the first and second maxillae (Fig. 13A).

At this point in the cycle, with the maxillae fully extended, there may be a pause of varying duration during which the first maxillae go through an independent set of movements. In these, contraction of the flexor muscles of the first maxillae causes the denticulate distal components to swing inward towards each other through an arc of 70–80° (Fig. 13A); the return movement outward is effected by contraction of the appropriate extensor fibers.

Return of the maxillae, carrier and dorsal rod to the resting position is effected by slight contraction of the carrier retractor muscles and appropriate actions by the other muscular components of the proboscis apparatus.

An uninterrupted cycle of proboscis movements (Figs. 7–11), without independent operation of the first maxillae, occupies on average 75 milliseconds (calculated from an analysis of movements filmed at 64 frames per second which showed that an average cycle occupied only 5 frames). This rate of approximately 12 cycles per second can be maintained for up to 5 seconds when the animal is feeding actively but more usually 2–3 seconds of activity are followed by resting periods varying in duration from a few seconds to many minutes. The pauses within a single cycle, with the maxillae fully extended, may last for 2–3 seconds during which the first maxillae perform their own independent movements at a rate of 10–11 per second.

The salivary glands. Nine pairs of unicellular salivary glands lie in the posterior half of the head (Fig. 15). The gland cells, labelled 1–9 in the Figure, are spherical to oval, 15–17 μm in diameter and produced anteriorly into long narrow ducts which open into the buccal cavity near the maxillae or into the oral groove. They fall into four groups as regards the staining reactions, point of discharge and role of their secretions.

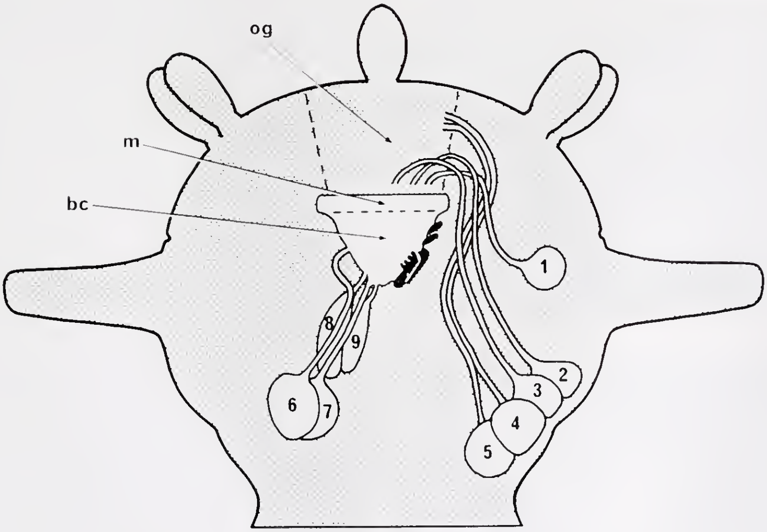
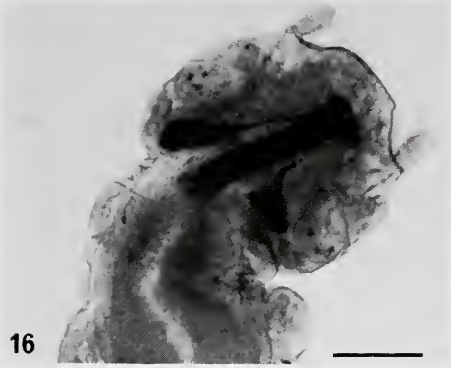
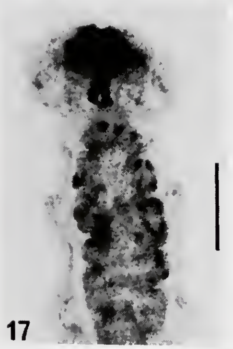
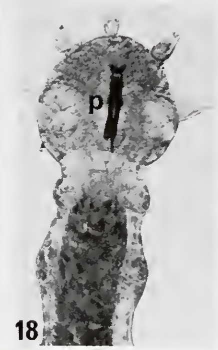
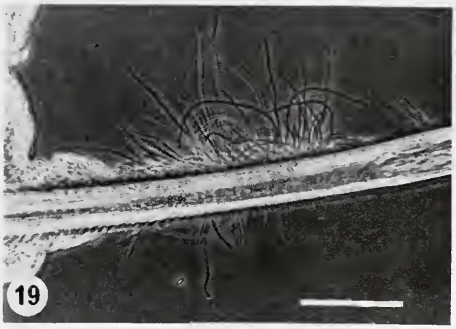


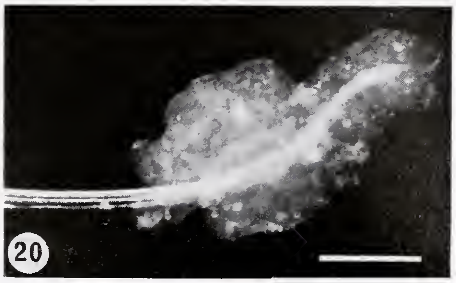
FIGURE 15. *H. homari.* Outline of the head from the dorsal aspect to illustrate the distribution of the salivary glands and their ducts. For clarity only one member of each pair of glands (labelled 1–9) is shown; bc, buccal cavity; m, mouth; og, position of oral groove on ventral surface of head.


16

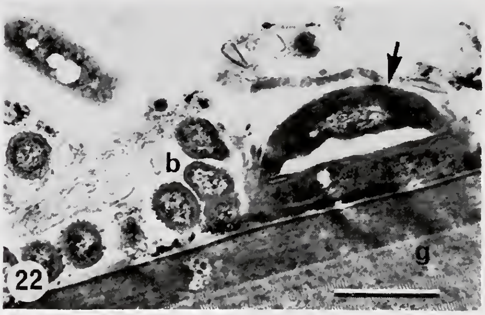

17

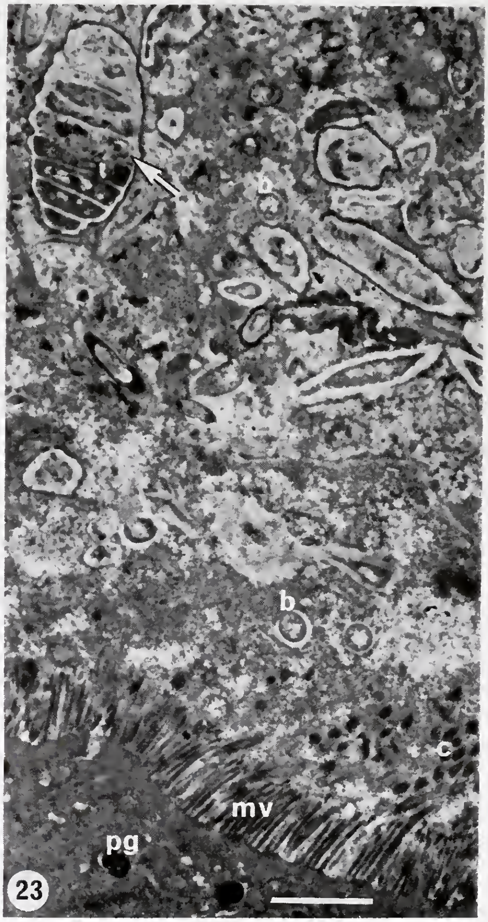

18


19


20

21


22


23

The secretions of cell pairs 1–5 stain weakly with Alcian blue and periodic acid-Schiff. They are discharged into the oral groove in front of the anterior lip and form the transport medium in which food particles are conveyed into the mouth. Secretions from cell pair 6, in contrast, stain very strongly with Alcian blue (Fig. 16) and periodic acid-Schiff, indicating a high acid mucopolysaccharide content which is probably mucin. They are discharged onto the exposed ridged surfaces of the second and third maxillae and bind together food particles as these are removed from the substratum.

The secretions of cell pair 7 show no reaction to Alcian blue but do stain strongly with periodic acid-Schiff, showing that they have a high carbohydrate content. They are discharged at the junction of the oesophagus and buccal cavity and join incoming food particles as these are drawn upward into the oesophagus.

Cell pairs 8 and 9 produce secretions negative to Alcian blue and periodic acid-Schiff but which give strong positive reactions for C-type esterases. These are identical with the reactions shown by the major component of the secretions produced by the gland cells of the stomach, which will be described later. The salivary esterases are discharged into the buccal cavity below the first maxillae

---

FIGURE 16. *H. homari.* The head and anterior body showing the sixth pair of salivary glands, flanking the proboscis, which produce the strongly Alcian blue positive mucous secretions. The whole mount preparation is stained with Alcian blue; scale is 50 μm.

FIGURE 17. Whole mount of *H. homari* showing concentrations of esterases in the gland cells of the stomach, with smaller amounts in the absorptive cells. The dark area in the head surrounding the anterior portion of the proboscis results from esterase activity around the brain and in the eighth and ninth pairs of salivary glands. The whole mount preparation was treated by Holt's method for nonspecific esterases; scale is 100 μm.

FIGURE 18. Whole mount of *H. homari* showing β-glucuronidase activity in the stomach wall. This specimen also demonstrates the prominence of the proboscis (p) as a feature of the head; during fixation the dorsal rod has been displaced posteriorly. The specimen was treated by Pearse's modification of the post-coupling method for β-glucuronidase; scale as in Figure 17.

FIGURE 19. Middle portion of an epipodite seta from *Homarus americanus* photographed by phase-contrast illumination and showing clusters of blue-green algae (center) and the edge of a mass of mucilage-secreting bacteria (left). Scale is 35 μm.

FIGURE 20. Distal portion of an epipodite seta from *Homarus vulgaris* showing a characteristically shaped mucilaginous mass containing numerous bacteria. Photographed by darkground illumination; scale is 75 μm.

FIGURE 21. Median portion of a seta from *Homarus vulgaris* showing fringing growths of colorless blue-green algae. The preparation is stained with Alcian blue; scale is 50 μm.

FIGURE 22. Electron micrograph of a section through a gill filament (g) from *H. americanus* showing, on the surface of the filament, profiles of bacteria (b), embedded in a mucilaginous matrix, and of two cells of a blue-green alga (arrowed). Fixation was in glutaraldehyde and osmium tetroxide (following Parke and Manton, 1967); the section is stained with uranyl acetate and lead citrate. Scale is 1 μm.

FIGURE 23. Electron micrograph of a section of *H. homari* fixed immediately after removal from *Homarus americanus*. The section shows the distal region of an absorptive cell in the stomach wall (bottom left) and a characteristic array of ingested microorganisms, contained within a granular matrix, in the stomach lumen. These include part of a filament of blue-green algal cells (arrowed) and bacteria (b). The surface of the absorptive cell bears cilia (c) and microvilli (mv), and two pigment granules (pg) can be seen within the cell. Preparation and staining of the section were the same as for Figure 22; scale is 2 μm.

and are poured onto incoming food particles before these are swept toward the oesophagus.

The oesophagus, proventriculus, stomach and intestine. The alimentary canal beyond the buccal cavity is an unbranched tube whose wall consists of an inner epithelium surrounded by thin layers of inner circular and outer longitudinal muscles. A detailed account of its ultrastructure will be given elsewhere; the present description is limited to basic features necessary for an understanding of the general pattern of digestive physiology.

The oesophagus ascends obliquely backward from the roof of the buccal cavity over the proboscis and joins the proventriculus in the posterior head region (Figs. 1A and 1B). The junction of oesophagus and proventriculus is marked by an internal constriction caused by an increase in the thickness of the lining epithelium; both the oesophagus and proventriculus are densely ciliated and the thickening of the epithelium at their junction results in an effective valvular arrangement of cilia which controls entry of material into the proventriculus.

The proventriculus is a relatively small chamber separated from the stomach by a muscular constriction at the point where the gut passes from the head into the first body segment. The muscular constriction results from thickening of the circular muscle of the gut wall at this point and controls entry of food into the stomach. The epithelium lining the proventriculus is similar to that of the oesophagus, consisting of ciliated cuboidal cells 8–10 μm tall and lacking any glandular components.

The stomach is the largest portion of the alimentary canal (Figs. 1A and 1B) and lies in body segments 1–5. It is expanded over most of its length into a voluminous chamber, almost filling segments 1–3, and tapers posteriorly as it passes over or between the gonads in the male and female, respectively. The anterior third of the chamber has thicker walls than the rest of the stomach, and its posterior limit is marked internally by a ring of very tall columnar cells. There is not, however, any modification of the musculature to form a sphincter and the ridge of cells appears to serve simply as a mechanical partial barrier, enhanced by the cilia of its cells, which contributes to retention of food in this anterior portion of the stomach.

The epithelial lining of the stomach is differentiated into glandular and absorptive cells which are deeply interdigitated with each other. The gland cells occur mainly in the anterior portion of the stomach, where 25–30% of the cells are of this type, but they also occur in smaller numbers posteriorly. They are conical cells 9–10 μm tall and 7–8 μm wide basally, with prominent nuclei. Their apices lack cilia but bear many short tightly packed microvilli. Ultrastructurally the cells are typical secretory structures, with the cisternae of the rough endoplasmic reticulum distended by large amounts of amorphous material. Histochemical techniques reveal that the cells produce organophosphate- and eserine-resistant esterases which are optimally demonstrated in the standard indoxyl acetate incubation medium at pH 4.5 (Fig. 17). The reaction is enhanced by inclusion in the medium of 10^{-3} M cysteine or 10^{-4} M sodium *p*-chloromercuribenzoate, and is 80–90% inhibited by inclusion of 10^{-2} M β-phenylpropionic acid (βPPA). This combination of properties indicates that a mixture of A- and C-esterases is present (Pearse, 1972) with the C-esterases (inhibited by βPPA) predominant. The esterases of the salivary secretions differ from these gastric esterases only in being totally inhibited by βPPA and appear, therefore, to consist exclusively of C-esterases.

The absorptive cells are cuboidal to trapezoidal, 9–10 μm tall, with large basal nuclei and prominent nucleoli. Their free distal surfaces are uniformly ciliated and bear regular rows of microvilli, 0.3–0.4 μm in length, between the cilia. They contain variable numbers of refractile brown to black pigment granules, 0.5–0.8 μm in diameter, which are insoluble in organic solvents and dilute mineral acids (Figs. 23 and 24). The cells also contain lipid globules which are of the same size range as the pigment granules and, like these, vary in number in different cells. There is, however, little apparent correlation between the numbers of pigment granules and lipid globules persent in any one cell.

Ultrastructurally the absorptive cells differ from the gland cells in that the cisternae of their rough endoplasmic reticulum are much narrower, with parallel walls and less prominent contents. A further difference is the presence in the cells of variable numbers of oval to spherical vesicles 0.4–0.8 μm in diameter which are filled with finely granular material. The pigment granules either occur within these vesicles (Fig. 24) or show remnants of them around their periphery. Small dense bodies, 0.1–0.3 μm in diameter and originating in the Golgi, also occur in the cells and may be found around the vesicles. Histochemical methods show that the absorptive cells produce A- and C-esterases, in smaller amounts than the gland cells but with C-esterases still predominating, β-glucuronidase (Fig. 18) and acid phosphatase. These enzymes were optimally visualized at pH 5.0 and, at the light microscope level, are localized in granules of approximately the same size and distribution as the dense bodies produced by the Golgi. It is concluded, therefore, that the bodies are lysosomes, although they consistently failed to give any reaction for arylamidases which are usual lysosomal constituents.

The only other enzyme demonstrated in the absorptive cells was alkaline phosphatase, which occurs in a narrow distal band 0.5–1.0 μm deep in the cytoplasm immediately below the ciliated distal surface. This zone also contains many mitochondria (Fig. 24) and the enzyme is probably, therefore, of mitochondrial origin.

The junction between the stomach and the intestine in segment 5 is marked by a ring of columnar cells similar to those at the junction of the anterior and posterior chambers of the stomach. The intestine (Figs. 1A and 1B) is narrower than the stomach and runs as a straight tube through segments 6, 7, and 8. It terminates at the anus in the mid-dorsal line on segment 9, between the two posterior adhesive organs formed by the bifurcation of this segment. Its epithelial lining varies in thickness from 4 μm to 8 μm, so that the luminal surface has a slightly corrugated appearance, and the constituent cells closely resemble the absorptive cells of the stomach. They are uniformly ciliated, with regular short microvilli between the cilia, and contain variable quantities of lipid globules and pigment granules. The latter, as in the absorptive cells, are generally enclosed within vesicles or the remnants of vesicles; lysosomes are associated with the vesicles and also occur throughout the cells. The rough endoplasmic reticulum is of the same type as that of the absorptive cells and mitochondria are common immediately below the ciliated distal surface. Only A- and C-esterases and acid phosphatase could be visualized histochemically in the lysosomes; no other enzymes could be demonstrated in the

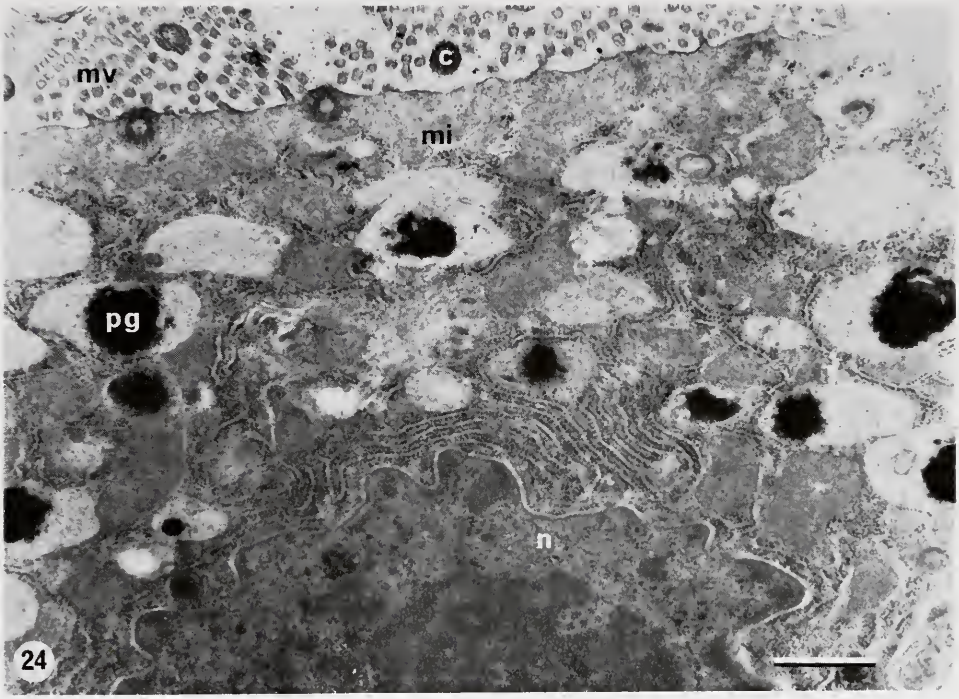


FIGURE 24. *H. homari.* Electron micrograph of a section through the distal half of an absorptive cell from the stomach wall. Abbreviations are: c, cilium; mi, mitochondria; mv, microvilli; n, nucleus; pg, pigment granule within a vesicle and surrounded by finely granular material; preparation as for Figure 22; scale is 1.5 μm.

intestinal cells apart from alkaline phosphatase which occurs in the distal regions of the cells surrounding the anus.

The circular muscle of the gut musculature is thickened around the anal opening to form a distinct sphincter, comparable to that surrounding the junction between the proventriculus and stomach.

The food and feeding mechanism

Forty lobsters were examined during this study (20 *Homarus americanus* in July and August 1973 and 1974, and 20 *H. vulgaris* from April to June and September to December 1974). All carried *H. homari* within the branchial chamber, the numbers present ranging from 5 in a soft-shelled, recently molted *H. americanus* to over 100 in several inter-molt specimens of both species. Every lobster, except for the soft-shelled specimen, also carried rich growths of micro-organisms on the inner surfaces of the branchial chamber, the setae fringing the edges of the carapace, the gill filaments and, especially, the surfaces and setae of the epipodite plates which lie between the gills.

The microfauna included many species of stalked and sessile ciliated protozoa, occasional small colonies of calyptoblastic hydroids such as *Clytia* sp., sessile and creeping species of rotifers and various adult and larval copepods. The microflora (Figs. 19–22) consisted principally of bacteria and blue-green algae, but some filamentous and spherical unicellular green algae were also present. The bacteria included unbranched chains of large Gram-negative rods, some of which were tentatively identified as members of the Flexibacteriales, and others, including both Gram-positive and Gram-negative rods, which were embedded in mucilaginous masses borne on various structures within the branchial cavity (Figs. 19, 20 and 22). The blue-green algae were generally growing in characteristic rosette-shaped groups of filaments (Figs. 19); they showed the usual blue-green coloration but colorless forms were also present either in rosettes or, more usually, as fringing filaments clothing many of the epipodite setae (Fig. 21).

Examination of the gut contents of over 100 *H. homari* showed that the polychaete feeds exclusively upon the microflora of its habitat, and no traces of materials referable either to the host or the microfauna were found in any part of the alimentary canal. Apart from this major discrimination between substances of plant and animal origin, however, *H. homari* appears to be a relatively unselective feeder and the proportions of bacteria, blue-green and green algae in the gut contents were much the same as in the habitat.

When the polychaete is about to feed, it establishes a secure hold upon the substratum with its posterior and anterior adhesive organs. At this stage the proboscis apparatus is in the resting position (Figs. 11 and 13B). The oral groove and mouth are placed over the food, the anterior lip is drawn forward and upward, and the posterior lip backward and downward. A slight forward movement of the head brings the exposed serrated edges of the mandibles into contact with the food, usually where it is attached to the substratum. The cycle of proboscidial movements, as described earlier, then begins. The maxillae move first posteriorly and then antero-laterally into their fully expanded positions (Figs. 7–10 and 13A). They are then pulled ventrally and inward toward the mid-line (Fig. 11) to complete the cycle. During this ventro-medial movement the toothed surfaces of the first maxillae and the ridged surfaces of the second, third and fourth maxillae draw the food, rake-like, across the serrated margins of the mandibles and detach it from its substratum.

Alternatively, the second, third and fourth maxillae may remain fully extended laterally, for a time, while the first maxillae operate independently, rapidly opposing their toothed surfaces and detaching the longer filamentous food organisms.

The secretions of the nine pairs of salivary glands (Fig. 15) are discharged in sequence during detachment and ingestion of the food. Secretions from the first five pairs of glands, which contain only a small proportion of mucus judging from their staining reactions, are the first to be discharged. They are poured from the roof of the oral groove onto the food as the maxillae commence their movements and are drawn through the buccal cavity into the oesophagus by the action of the oesophageal cilia which are particularly long and densely packed at the junction of the oesophagus and buccal cavity. The secretions from the sixth pair of glands, containing a much larger proportion of mucus, are released as the maxillae detach food particles. They cause the detached particles to adhere together in irregular clumps which are then swept with the secretions from the first five pairs of glands into the oesophagus. As the particles pass through the buccal cavity they receive the esterase-rich secretions from the eighth and ninth pairs of salivary glands, and, at the point of entry to the oesophagus, they receive the periodic acid-Schiff positive secretions from the seventh pair.

The clumps of food particles and the various salivary secretions are swept up the oesophagus into the proventriculus where they remain until this chamber is full, onward passage into the stomach being prevented by contraction of the sphincter at the proventricular-stomach junction, while the valvular arrangement of the cilia at the proventricular-oesophageal junction prevents return down the oesophagus. During their stay in the proventriculus, which may occupy 1–30 seconds depending on the rate of feeding, the food organisms and salivary secretions are rotated and thoroughly mixed by ciliary action. Eventually the sphincter relaxes briefly, and the contents of the proventriculus are immediately swept into the stomach.

Digestion

Materials entering the stomach consistently show small amounts of A- and C-esterase activity caused by the intrinsic enzymes of the food organisms and the C-esterases contributed by the salivary secretions. The level of esterase activity, however, rises rapidly as the gland cells of the stomach discharge their secretions, which consist mainly of C-esterases. The stomach contents are kept in continuous movement by the cilia of the absorptive cells; this results initially in the efficient mixing of the fluid and solid components during which there is presumably a considerable amount of digestion since many of the ingested microorganisms lose their identity. Bacteria progressively disappear, blue-green algae lose their cellular contents and the granular matrix in which the food organisms are ingested, formed from the mucous salivary secretions and the masses of mucilage surrounding some of the bacteria, becomes more homogeneous (Fig. 23).

The continuous rotation of the stomach contents eventually causes aggregation of solid particles, some of which are still undergoing digestion, into a number of strings. This occurs in the anterior stomach and is particularly noticeable when the ingested food contains a large proportion of mucilaginous bacterial colonies. The strings gradually accumulate in the posterior stomach where they coalesce into oval pellets, 50–100 μm long and 10–20 μm wide. Formation of the strings and pellets effectively separates the solid from the fluid contents of the stomach; the separation is then rapidly followed by progressive decrease in the volume of the fluid component. This is caused by absorption of some of the fluid by the absorptive cells and the passage of the remainder into the intestine where it too is absorbed. Disappearance of fluid from the stomach and intestinal lumina is accompanied by development of vesicles in the epithelial cells (Fig. 24); the contents of these vesicles have the same appearance and staining reactions (faintly acidophilic and Alcian blue and periodic acid-Schiff positive) as the fluid materials in the gut lumen and presumably consist of the digested and semidigested products of the extracellular digestion effected by the esterases from the salivary glands and stomach gland cells. The method of uptake by the epithelial cells is unknown, as no evidence of phagocytosis or pinocytosis was found. It is concluded that materials enter by some type of absorptive process and then collect to form the intracellular vesicles; the abundance of mitochondria in the distal regions of the cells suggests that this is probably an active, energy-dependent process.

The association with the vesicles of lysosomes containing A- and C-esterases, acid phosphatase and, in the stomach cells, β-glucuronidase, and the occurrence of pigment granules in the vesicles indicates that digestion is completed within these and that the pigment granules are accumulated insoluble end-products.

The intracellular digestion occurring in the intestinal epithelium differs somewhat from that in the stomach absorptive cells in that β-glucuronidase is not involved. Further, the A- and C-esterases demonstrated in the intestinal lysosomes were optimally visualized at pH 7.0–7.5, as compared with pH 5.0 for those in the stomach cells. There must thus be differential absorption in the stomach and intestine of substances whose digestion requires, in its later stages, different enzymes and different pH optima.

Intra-vital staining of *H. homari* with various indicators showed that the pH of the food drops sharply to between pH 4.0 and 5.0 as it enters the stomach, and it remains at this level throughout its stay in this organ. The absorptive cells showed a similar pH value, in those instances where staining of the cells was adequate, and these observations support the histochemical indications that both extra- and intracellular phases of digestion in the stomach occur in an acidic medium. The intestinal cells showed a pH value of between 7.0 and 8.0 and, again, this agrees with the histochemical findings that intracellular digestion in the intestine proceeds in a slightly alkaline medium.

The pellets of indigestible residues eventually pass into the intestine and are then rapidly swept to the anus and expelled. The intracellular residues, however, do not appear to be voided into the gut lumen and are believed to remain in the stomach and intestinal cells throughout life. This conclusion is supported by the absence of pigment granules from the gut of newly hatched *H. homari* and observation of increasing amounts in immature through sexually mature adults.

Movement of food through the alimentary canal is effected primarily by ciliary action; the gut musculature is not especially well developed and does not cause any significant peristaltic movements of the food.

Food reserves

Lipid forms the principal food reserve of *H. homari*. Large quantities occur in the absorptive cells of the stomach and in the intestinal epithelium as globules 0.5–0.8 μm in diameter. These reserves are rapidly depleted if the polychaete is kept away from its host in filtered sea water and disappear after 3–4 days. Their depletion is quickly followed by death, and no isolated individuals survived for longer than five days.

Very small amounts of glycogen occur in the same cells as the lipid reserves and also in the gonads. These disappear within hours of deprivation of food and do not constitute significant long term reserves.

DISCUSSION

These observations show that the relationship between *Histriobdella homari* and its crustacean host has a firm nutritional basis, with the polychaete feeding exclusively on the microflora of the lobster's branchial chamber. The relationship is thus not detrimental to the host but is, in fact, probably beneficial in that removal of encrusting microorganisms from respiratory structures can only be advantageous. Total removal, of course, effectively occurs when the host molts, but continuous small-scale removal by *H. homari* during inter-molt periods probably prevents excessive build-up of microorganisms. *H. homari,* therefore, can be regarded as an epizoic microphagous cleaning symbiote.

The variety and quantity of the microfloral growths consistently found in the lobster branchial chamber was somewhat surprising. Epizoic blue-green algae have been reported from some other crustacea (Margalef, 1953; Bunting and Lund, 1956; Shelton, 1974) but not in the quantities observed in the present study or in association with other microorganisms; the microflora and fauna of the lobster branchial chamber constitute an interesting and compact ecosystem which merits further study. In the present context, the microflora is seen to provide a rich food source for *H. homari* and its abundance in the specimens examined was probably a major factor contributing to the 100% incidence and high individual host infestation rate of the polychaete. Similar incidence and infestation rates have been recorded, for *Homarus americanus,* by Uzmann (1967) and Simon (1967; 1968) and *H. homari,* therefore, appears to enjoy a considerable degree of success as a symbiote.

The diet, digestive physiology and food reserves of *H. homari* are very similar to those of some other polychaetes which are microfloral grazers (Jennings and Gelder, 1969; Gelder and Uglow, 1973) and appear to be virtually unaffected by adoption of the symbiotic habit. The range of digestive enzymes present is somewhat limited and lacks, for example, exopeptidases which are easily demonstrable, by the arylamidase technique, in carnivorous and sanguinivorous annelids (Jennings and van der Lande, 1967). There is, though, considerable emphasis on production of β-glucuronidase, and both these features are clearly adaptations to the nature of the diet rather than to the mode of life. A comparable situation occurs, for example, in the free-living nematode *Monhystera denticulata* which, like *H. homari,* is microphagous with a high proportion of bacteria in its diet, lacks intestinal exopeptidases, but produces considerable quantities of β-glucuronidase (Jennings and Deutsch, 1975).

The principal adaptive feature in the nutrition of *H. homari,* then, would seem to be in the proboscidial apparatus which is very much modified from the basic eunicid pattern and is more complicated in structure and mode of operation than the feeding mechanisms of most other polychaete microfloral grazers. A possible reason for this is that while the food organisms utilized are of the same type as those taken by free-living grazers their substrata are very different and they are probably more firmly attached to them as an adaptation to the constant flow of water through the host's branchial chamber. Thus, the initial dislodging of the food organisms prior to ingestion may require either greater force or a more specialized reaping-type of mechanism than would be needed to dislodge similar epiphytic or epilithic organisms.

Modification of the eunicid proboscis into the histriobdellid form has involved development of the maxillae into articulated structures, elaboration of the transverse carrier into a vehicle capable of controlled anterior and posterior movement, the use of the dorsal rod (which is probably a modified backward prolongation of the carrier) to store energy for protraction of the maxillae, and the separation and development of the muscular components. These modifications allow the now-movable maxillae to be used in conjunction with the fixed mandibles as a reaping mechanism which detaches algae and bacteria from their substrata for subsequent ingestion by ciliary action.

The eunicid proboscis appears to have had the potential to evolve in a number of different ways (*vide* Dales, 1962) and the histriobdellid type, as seen in *H homari,* is probably the most elaborate form to have arisen. A comparable modification, but along different lines and resulting in movable scissor-like mandibles, has occurred in the family Ichthyotomidae which contains the single species *Ichthyotomos sanguinarius.* This species, too, is symbiotic but appears to be ectoparasitic rather than epizoic; it lives on eels attached to the gills or fins and uses the mandibles for adhesion and to release blood which is then ingested (Eisig, 1906).

Modification of the proboscis in *H. homari* has been accompanied by specialization of the salivary glands which have become important components of the feeding mechanism, with their secretions performing at least three distinct functions. One of these is to trap microorganisms dislodged by the maxillae and prevent their being swept away by the host's respiratory current, a second is to provide a transport medium for the food and the third is to initiate digestion.

Accounts of other members of the Histriobdellidae by Haswell (1913), Cordero (1927), Lang (1950) and Roubaud (1962), when re-examined in the light of the present findings, indicate that their relationships with their respective hosts are much the same as between *H. homari* and its hosts and that the pattern of nutritional physiology seen in this species is probably characteristic of the entire family. If this is correct, then the Histriobdellidae, as symbiotic polychaetes, are directly comparable to the Temnocephalida which are symbiotic Turbellaria whose general pattern of nutrition is virtually the same as that of their free-living relatives (Jennings, 1971). Both groups live epizoically in the branchial chamber of decapod crustaceans, the histriobdellids as microfloral grazers and the temnocephalids as carnivores. The latter prey, in part, on animals living epizoically in the same habitat and which, therefore, are analogous to the food organisms utilized by the histriobdellids.

A further interesting parallel between the Histriobdellidae and Temnocephalida is seen in their geographical distribution. The temnocephalid-crustacean symbioses occur in fresh water in Australasia, Madagascar and South and Central America; the histriobdellid-crustacean associations have the same distribution apart from the aberrant occurrence of *H. homari* on marine decapods in Northwest Europe and Northeast America. With regard to this last point, it is noteworthy that both temnocephalids and histriobdellids are typical freshwater organisms in that they have eliminated free-swimming ciliated larval stages from their life cycles and hatch as miniature immature adults. This suggests that *H. homari* has at some time become secondarily adapted to the marine habitat, as also must have *Stratiodrilus cirolanae* Führ 1971 which is the only other marine histriobdellid so far recorded.

The geographical distribution of the histriobdellids and temnocephalids indicates a very ancient origin for their associations with crustaceans and, therefore, that the type of relationships in which the modern members of the two groups are involved probably represent end-points in the evolution of these particular symbioses.

The close parallels in geographical distribution and host types have resulted in some instances in the occurrence of both histriobdellids and temnocephalids on the same individual hosts. *Stratiodrilus tasmanicus* and *Temnocephala quadricornis,* for example, have been found together in the branchial chamber of *Astacopsis tasmanicus* in Tasmania (Haswell, 1900); while *S. platensis* and *T. chilensis* have been recorded from *Aeglea laevis* in Uruguay (Cordero, 1927; Roubaud, 1962). It would be interesting to know whether the *Stratiodrilus* are preyed on by the temnocephalids; if this does occur and the *Stratiodrilus* are feeding on the same type of organisms as *H. homari,* then the branchial chambers of the host crustaceans must indeed contain a complex ecosystem, with components ranging from primary producers to metazoan carnivores.

This work was supported by a U.K. Science Research Council grant (No. 2016/4) to J.B.J.; it was carried out mainly at the University of Leeds, England but in part at the Marine Science Institute, Nahant, Massachusetts, where S.R.G. was supported by an honorarium from that Institute; and at the Marine Biological Laboratory, Woods Hole, Massachusetts in association with the Experimental Invertebrate Zoology Course in 1973 and 1974. We wish to thank Dr. M. Patricia Morse and Dr. Nathan W. Riser, of the M.S.I., Nahant, for hospitality and for drawing our attention to this interesting polychaete.

SUMMARY

1. The aberrant annelid *Histriobdella homari* (Polychaeta:Eunicida) lives in the branchial chambers of the marine lobsters *Homarus americanus* and *H. vulgaris* where it feeds on the rich microflora of bacteria, blue-green algae and related organisms which grow on the inner surface of the branchial chamber, the setae fringing the edges of the carapace, the gill filaments and, especially, the surfaces and setae of the epipodite plates between the gills. *H. homari,* therefore, is to be regarded as an epizoic microphagous cleaning symbiote of the lobsters.

2. The alimentary canal consists of mouth, buccal cavity, oesophagus, proventriculus, stomach, intestine and anus. The much-modified proboscis lies ventrally below the oesophagus and proventriculus, with its anterior portions protruding into the rear of the buccal cavity.

3. The proboscis consists of two fixed parallel mandibles, a transverse carrier which slides upon the mandibles and to which is attached, posteriorly, a median flexible dorsal rod and, anteriorly, four pairs of movable articulated maxillae, paired external and internal retractor muscles and various tensor, flexor and extensor muscles.

4. Contraction of the retractor muscles withdraws the carrier and maxillae posteriorly, causing bowing of the dorsal rod which is fixed at its posterior end. Relaxation of the muscles allows the rod to straighten and, thus, causes protraction of the carrier and protraction and lateral expansion of the maxillae. Contraction and relaxation of the retractor muscles are supplemented by appropriate changes in the other muscular components of the proboscis.

5. During feeding the serrated anterior ends of the mandibles are applied to the food, the maxillae are fully expanded and then drawn ventro-posteriorly toward the mid-line by contraction of the retractor muscles in the effective movement of the feeding mechanism. This draws the food organisms across the anterior ends of the mandibles, detaching them from the substratum and allowing their ingestion by ciliary action. The first pair of maxillae are also capable of independent action and can be used while the remainder of the proboscis apparatus is held in the protracted position.

6. Detached microorganisms are entangled in a sticky mucous secretion from the salivary glands; other salivary secretions provide a transport medium for the clumped particles and a third set contain C-esterases which initiate digestion.

7. Ingested food is held briefly in the proventriculus, then passed to the stomach where gland cells secrete A- and C-esterases which continue and extend the digestion initiated by the salivary C-esterases.

8. Some soluble products of gastric digestion are taken up by absorptive cells in the stomach wall and their digestion is completed intracellularly by enzymes which include β-glucuronidase. Others pass into the intestine for absorption and completion of digestion by cells similar to the gastric absorptive cells but which lack β-glucuronidase. Insoluble residues of intracellular digestion accumulate in the stomach and intestinal cells as pigmented granules; residues of extracellular digestion aggregate in pellets and are voided through the anus.

9. Lipid forms the principal food reserve and is laid down in the absorptive cells of the stomach and intestine.

10. The histriobdellid-crustacean type of symbiosis, as exemplified by *H. homari* and its lobster hosts, is compared with the temnocephalid (Platyhelminthes: Turbellaria)-crustacean type. Basic similarities in the relative lack of specialization in the nutritional physiology of the annelid and platyhelminth symbiotes, when compared with that of their free-living relatives, are discussed, as also are the implications of known similarities in their life histories and geographical distributions.

LITERATURE CITED

Beneden, P. J. Van, 1853. Note sur une larve d'annélide d'une forme tout particuliere, rapportée avec doute aux serpules. *Bull. Acad. Roy. Sci. Let. Beaux-Arts Belg.,* **10**: 69–72.

Beneden, P. J. Van, 1858. Histoire naturelle d'un animal nouveau, désigné sous le nom d'*Histriobdella. Bull. Acad. Roy. Sci. Let. Beaux-Arts Belg., 2me Ser.,* **5**: 270–303.

Bunting, W., and J. W. G. Lund, 1956. A new blue-green alga epizooic on *Daphnia pulex* L. *Naturalist,* **858**: 88–90.

Burstone, M. S., 1958. Histochemical demonstration of acid phosphatase with naphthol AS-phosphates. *J. Nat. Cancer Inst.,* **21**: 523–539.

Burstone, M. S., and J. E. Folk, 1956. Histochemical demonstration of aminopeptidase. *J. Histochem. Cytochem.,* **4**: 217–226.

Calow, P., and J. B. Jennings, 1974. Calorific values in the phylum Platyhelminthes: the relationship between potential energy, mode of life and the evolution of entoparasitism. *Biol. Bull.,* **147**: 81–94.

Clark, R. B., 1956. *Capitella capitata* as a commensal, with a bibliography of parasitism and commensalism in the polychaetes. *Ann. Mag. Natur. Hist., Ser. 12,* **9**: 433–448.

Cordero, E. H., 1927. Un nuevo Arquianelida, *Stratiodrilus platensis* sp. n. que habita sobre *Aeglea laevis* (Latr.). Nota preliminar. *Sociedad Physis para el cultivo y difusión de las ciencias naturales en la Argentina,* **8**: 574–578.

DALES, R. P., 1962. The polychaete stomodeum and the interrelationships of the families of Polychaeta. *Proc. Zool. Soc. London,* **139**: 389–428.

DALES, R. P., 1967. *Annelids,* 2nd. ed. Hutchinson and Co. Ltd., London, 200 pp.

EISIG, H., 1906. *Ichthyotomus sanguinarius* eine auf Aalen Schmarotzende Annelide. *Fauna Flora Golf. Neapel.,* **28**: 1–300.

FAUVEL, P., 1959. Classe des Annélides Polychètes. Pages 13–196 in Pierre-P. Grassé, Ed., *Traité de Zoologie. Anatomie, Systématique, Biologie.* Tome V. Masson et Cie, Paris.

FOETTINGER, A., 1884. Recherches sur l'organisation de *Histriobdella homari* P. J. van Beneden rapportée aux Archiannélides. *Arch. Biol., Liége,* **5**: 435–516.

FÜHR, I. M., 1971. A new histriobdellid on a marine isopod from South Africa. *S. Afr. J. Sci.,* **67**: 325–326.

GELDER, S. R., AND J. B. JENNINGS, 1975. The nervous system of the aberrant symbiotic polychaete *Histriobdella homari* and its implications for the taxonomic position of the Histriobdellidae. *Zool. Anz.,* **194**: 293–304.

GELDER, S. R., AND R. F. UGLOW, 1973. Feeding and gut structure in *Nerilla antennata* (Annelida: Archiannelida). *J. Zool., London,* **171**: 225-237.

HARRISON, L., 1928. On the genus *Stratiodrilus* (Archiannelida: Histriobdellidae) with a description of a new species from Madagascar. *Rec. Aust. Mus.,* **16**: 116–121.

HASSALL, M., AND J. B. JENNINGS, 1975. Adaptive features of gut structure and digestive physiology in the terrestrial isopod *Philoscia muscorum* (Scopoli) 1763. *Biol. Bull.,* **149**: 348–364.

HASWELL, W. A., 1900. On a new histriobdellid. *Quart. J. Microsc. Sci.,* **43**: 299-335.

HASWELL, W. A., 1913. Notes on the Histriobdellidae. *Quart. J. Microsc. Sci.,* **59**: 197–226.

HERMANS, C. O., 1969. The systematic position of the Archiannelida. *Syst. Zool.,* **18**: 85–102.

HOLT, S. J., 1958. Studies in enzyme histochemistry. *Proc. Roy. Soc. London Ser. B,* **148**: 465–532.

HYMAN, L. H., 1951. *The Invertebrates: Platyhelminthes and Rhynchocoela. The acoelomate Bilateria,* Volume II. McGraw-Hill Book Co., New York, 550 pp.

JENNINGS, J. B., 1969. Ultrastructural observations on the phagocytic uptake of food materials by the ciliated cells of the rhynchocoelan intestine. *Biol. Bull.,* **137**: 476–485.

JENNINGS, J. B., 1971. Parasitism and commensalism in the Turbellaria. Pages 1–32 in B. Dawes, Ed., *Advances in parasitology,* Volume IX. Academic Press, New York.

JENNINGS, J. B., 1973. Symbioses in the Turbellaria and their implications in studies on the evolution of parasitism. Pages 127–160 in Winona B. Vernberg, Ed., *Symbiosis in the sea.* University of South Carolina Press, Columbia, South Carolina.

JENNINGS, J. B., AND A. DEUTSCH, 1975. Occurrence and possible adaptive significance of β-glucuronidase and arylamidase ("leucine aminopeptidase") activity in two species of marine nematodes. *Comp. Biochem. Physiol.,* **52A**: 611–614.

JENNINGS, J. B., AND S. R. GELDER, 1969. Feeding and digestion in *Dinophilus gyrociliatus* (Annelida: Archiannelida). *J. Zool. London,* **158**: 441–451.

JENNINGS, J. B., AND V. M. VAN DER LANDE, 1967. Histochemical and bacteriological studies on digestion in nine species of leeches (Annelida: Hirudinea). *Biol. Bull.,* **133**: 166–183.

JEON, K. W., 1965. Simple method for staining and preserving epoxy resin-embedded animal tissue sections for light microscopy. *Life Sci.,* **4**: 1839–1842.

LANG, K., 1950. A contribution to the morphology of *Stratiodrilus platensis* Cordero (Histriobdellidae). *Ark. Zool.,* **42A**: 1–30.

MARGALEF, R., 1953. Materiales para una flora de las algas del N. E. de España IVb, Cyanophyceae. *Collectanea botanica a Barcinonensi Botanico Instituto edita,* **3**: 231–260.

MESNIL, F., AND M. CAULLERY, 1922. L'appareil maxillaire d'*Histriobdella homari;* affinitiés des Histriobdellides avec les Euniciens. *C. R. Hebd. Séanc. Acad. Sci. Paris,* **174**: 913–917.

PARKE, M., AND I. MANTON, 1967. The specific identity of the algal symbiont in *Convoluta roscoffensis. J. Mar. Biol. Ass. U. K.,* **47**: 445–464.

PEARSE, A. G. E., 1972. *Histochemistry: theoretical and applied,* 3rd ed. Churchill Livingstone, Edinburgh and London, 1518 pp.

REMANE, A., 1932. Archiannelida. Pages 1-36 in J. G. Grimpe, Ed., *Die Tierwelt der Nord- und Ostsee,* 22 (Teil 6a).

ROUBAUD, G., 1962. Recherches sur les *Stratiodrilus platensis* Cordero, Archiannélides parasites des *Aeglea* des lacs de Patagonie. *Biologie de l'Amerique Australe* (*Paris*), **2**: 31–54.

SHEARER, C., 1910. On the anatomy of *Histriobdella homari. Quart. J. Microsc. Sci.,* **55**: 287–359.

SHELTON, R. G. J., 1974. Observations on the occurrence of an epizooic blue-green alga on the chemoreceptor setae of the brown shrimp, *Crangon crangon* (L.) *J. Mar. Biol. Ass. U. K.,* **54**: 301-307.

SIMON, J. L., 1967. Behavioral aspects of *Histriobdella homari,* an annelid commensal of the American lobster. *Biol. Bull.,* **133**: 450.

SIMON, J. L., 1968. Incidence and behavior of *Histriobdella homari* (Annelida; Polychaeta), a commensal of the American lobster. *Bioscience,* **18**: 35–36.

UZMANN, J. R., 1967. *Histriobdella homari* (Annelida: Polychaeta) in the American lobster *Homarus americanus. J. Parasitol.,* **53**: 210–211.

VAILLANT, L., 1890. *Histoire naturelle des annelés marins et d'eau douce: lombriciens, hirudiniens, bdellomorphes, teretulariens et planariens.* 3 Roret, Paris, 539 pp.

Reference: *Biol. Bull.* **151**: 518-537. (December, 1976)

BEHAVIOR OF THE SEA PANSY *RENILLA KOLLIKERI* PFEFFER (COELENTERATA: PENNATULACEA) AND ITS INFLUENCE ON THE DISTRIBUTION AND BIOLOGICAL INTERACTIONS OF THE SPECIES[1]

JON KASTENDIEK[2]

Department of Biology, University of California, Los Angeles, California 90024

This paper describes various behavioral patterns of the sea pansy, *Renilla kollikeri* Pfeffer (Alcyonaria: Pennatulacea), and their influences upon the species' distribution and biological interactions. The study of cnidarian behavior has long focused on patterns associated with either locomotion or feeding (reviewed in Mackie, 1974). However, during the last twenty years the predator-escape behavior of certain actinians has been examined (reviewed in Ross, 1974), and more recently, intraspecific and interspecific "aggression" among species of Actinaria has also been described (Francis, 1973; Lang, 1971, 1973). Few studies, however, have surveyed the behavioral repertoire of a single species and analyzed its role in determining the species' distribution and predator-prey interactions.

Previous work on pennatulacean behavior is limited. Recent papers describe burrowing in the genera *Pteroides* and *Veretillum* (Titschack, 1968; Buisson, 1971, 1974), the diurnal activity patterns of *Cavernularia obesa* (Mori, 1960) and *Scytaliopsis djiboutiensis* (Magnus, 1966), and the predator interactions of *Ptilosarcus guerneyi* (Birkeland, 1974). The only reports which discuss the behavior of the sea pansy are physiological in nature. These reports discuss muscular movement and colonial coordination (Parker, 1919, 1920b; Anderson and Case, 1975), water movement (Parker, 1920a), respiration (Chapman, 1972) and bioluminescence (Bertsch, 1968; Buck, 1973; Anderson and Case, 1975).

Renilla kollikeri is an abundant member of the shallow, sand bottom fauna of the southern California coast. It inhabits regions of strong turbulence. In sandy, nearshore subtidal areas (to depths of 10 meters), the drag of the ocean swell generates strong, multidirectional bottom currents (velocities of more than four meters per second have been recorded at a depth of three meters). This back-and-forth wave surge, which strengthens as depth decreases, is strong enough to suspend and move a layer of sand to and fro along the bottom. This movement not only buffets the fauna but buries animals under shifting sands. *Renilla* displays many morphological and behavioral features adaptive for life in this turbulent habitat.

The behavioral patterns described and experimentally analyzed below are concerned with three broad aspects of the biology of the sea pansy. First, the maintenance of position on the bottom, particularly the responses to increased water movement, burial by shifting sediment, and displacement from the substrate are discussed. Secondly, the species-specific escape behavior from the nudibranch, *Armina californica,* and other predator interactions are described; and thirdly, the food and mode of feeding of the colony are considered. These behavioral patterns are particularly adaptive to and influential in the local distribution of the species.

---

[1] This paper is based on a portion of a dissertation submitted to the University of California, Los Angeles, in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Biology. The work was supported in part by a National Science Foundation Pre-Doctoral Traineeship and by USPHS Grant 9546 awarded to Dr. James G. Morin.

[2] Present address: Department of Biological Sciences, University of Southern California, Los Angeles, California 90007.

MATERIALS AND METHODS

Field observations and experiments were conducted using SCUBA equipment in water depths of 3 to 15 meters. The primary study site was at Zuma Beach, Los Angeles County, California, a 6 kilometer long, southwest-facing sand beach, 35 kilometers northwest of Los Angeles, California. The beach is exposed to the open ocean and the surf ranges from 0.3 to 4.0 meters in height throughout the year. Comparative observations were made at Santa Barbara Harbor, Santa Barbara County and at Scripps Beach, San Diego County, California. Laboratory observations and experiments were conducted at the University of California, Los Angeles, and the Santa Catalina Marine Biological Laboratory. The experimental animals were collected by hand and maintained at ambient sea temperatures in both closed (UCLA) and open (SCMBL) circulating seawater systems.

The effects of water flow on *Renilla* were studied in both field and laboratory. In the laboratory animals were allowed to anchor in a layer of coarse sand in various sized aquaria. A water current was directed across the upper surface of the colony from a plastic tube 1.3 cm in diameter. Velocities were measured with

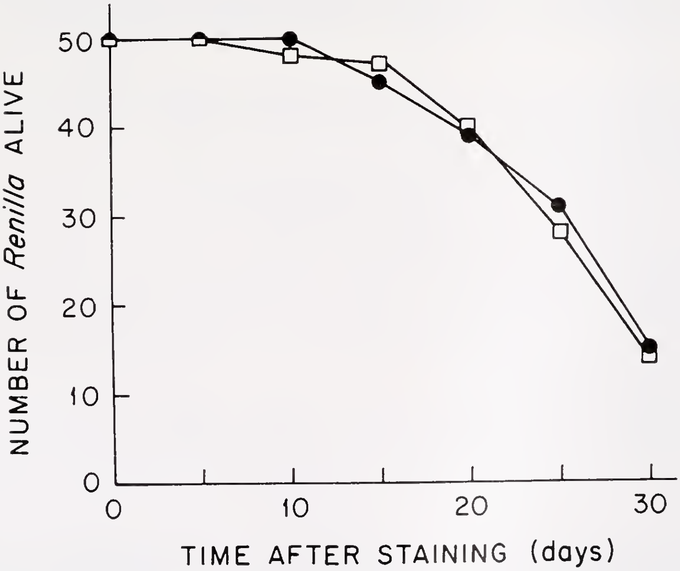


FIGURE 1. Laboratory survival of dyed (boxes) and undyed (closed circles) *R. kollikeri.* The animals were maintained at 13° C. No significant difference in survivorship was observed ($P > 0.5$; chi-square test).

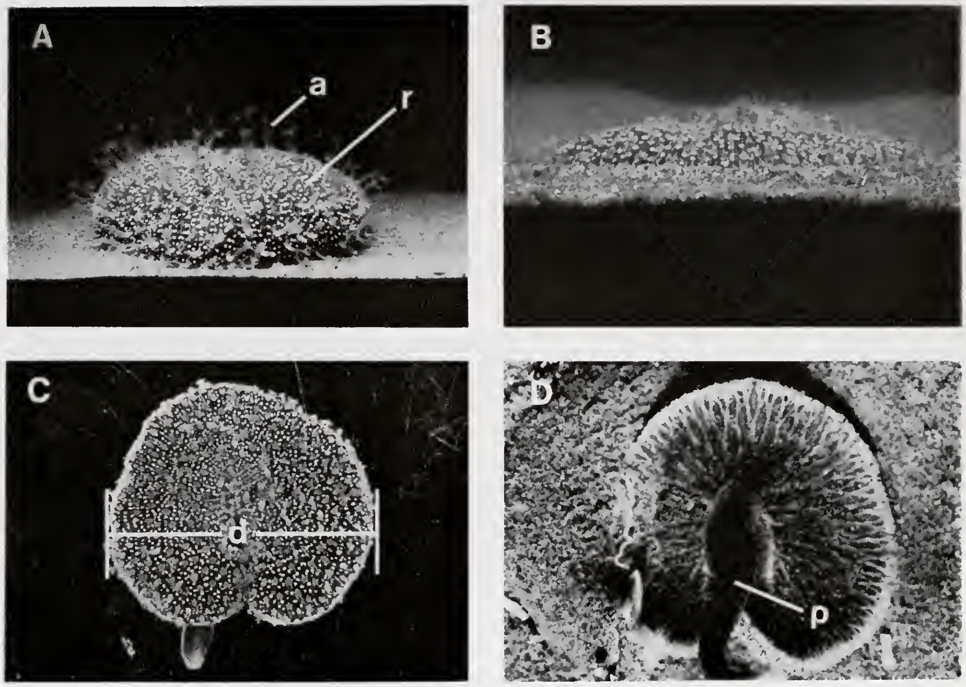


Figure 2. *Renilla kollikeri:* A. An expanded colony in still water. Note expanded autozooids (a). B. Deflated rachis characteristic of colony when in moving water and while reanchoring. C. Dorsal surface of rachis showing measure of diameter (d). D. Ventral surface showing peduncle (p). The diameter of the *Renilla* depicted is 65 mm.

a calibrated bead flowmeter (Kontes Flow-Watchman). Field water velocities were determined with a hand-held flow meter (Kahl Scientific Instruments Corporation: Model 005WA120).

Individuals were marked for field study by staining with the vital dye Nile Blue Sulfate. Animals were placed in a 5.0 per cent solution of dye in sea water for no more than three to five minutes. In high concentration or after prolonged exposure, the dye is caustic to the animals. However, stained *Renilla* exhibited the same survival over a two month period in the laboratory as unstained animals (Fig. 1). Furthermore, stained animals were in excellent condition five months after release in the field.

Field movement experiments lasted from six hours to two months. Displacement of dyed animals was measured with a polypropylene line marked in meters from a reference stake driven into the sand at the center of the released group.

All feeding experiments were conducted in the laboratory. Suspensions of potential prey items ranging in size and form from unicellular algae (30 to 50 microns in length) to *Artemia* nauplii and copepods (0.1 to 0.4 mm in length) were pipetted into a chamber containing a sea pansy. Subsequent interactions between predator and prey were observed at low light levels through a dissecting microscope.

The following anatomical terms will be used throughout the paper. The principal body regions of *Renilla* are the rachis and the peduncle. When a colony is anchored on the bottom, the rachis lies flush against the substrate (Fig. 2a, b). Two different types of zooids are interspersed on its dorsal surface: polypoid autozooids, which function in feeding and reproduction, and siphonozooids, which function in irrigating the colony. Rachis height, or thickness, is the maximum distance between the dorsal and ventral surfaces. Rachis diameter (or size) is the distance across the rachis at right angles to the median line (Fig. 2c). The peduncle is a column of tissue which projects from the ventral surface of the rachis and anchors the colony when it is inserted into the sand. When anchored, the tip of the peduncle is generally bulbous.

OBSERVATIONS AND RESULTS

Maintenance of position

The rachis. The rachis alters its degree of inflation (here defined as height/diameter) in an inverse relation to the velocity of water flowing over it (Fig. 3).

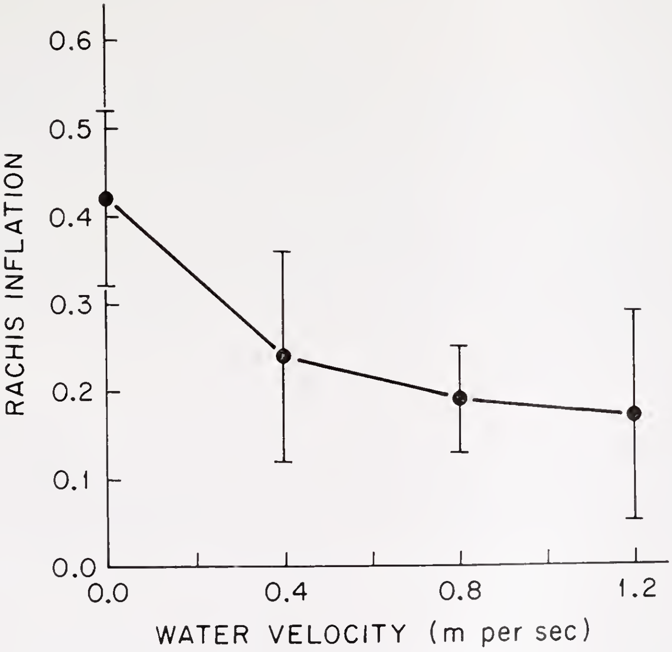


FIGURE 3. Inflation of rachis with respect to velocity of water flow. Inflation is ratio of rachis height to rachis diameter. Samples consisted of 40 animals which ranged from 20 to 90 mm in diameter and were exposed to flow for 30 minutes. There was a significant difference in the means of the samples ($P < 0.01$; ANOVA). Vertical bars represent 95 per cent confidence intervals.

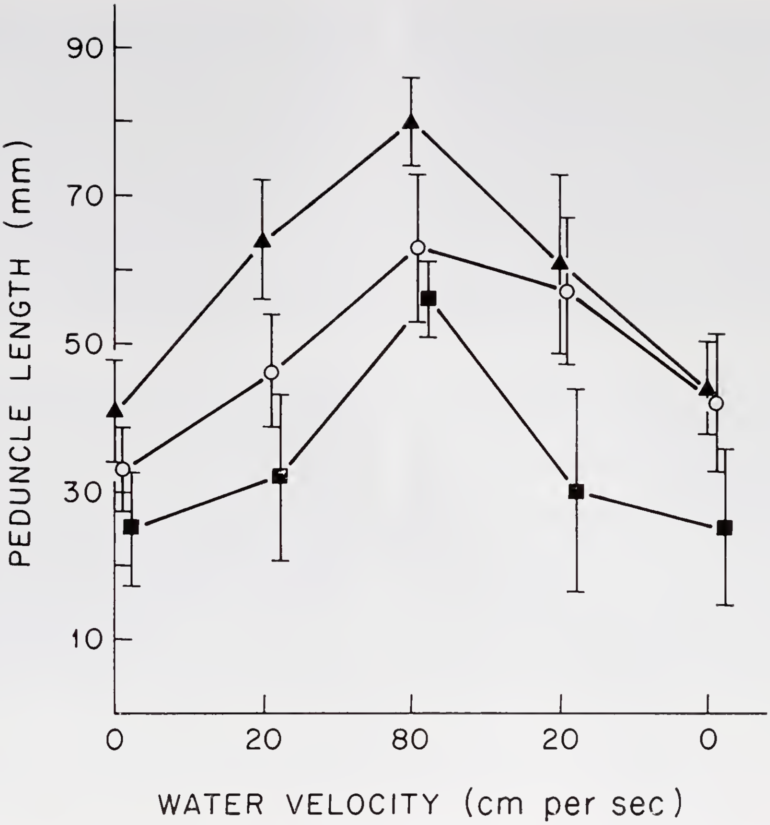


FIGURE 4. Peduncle insertion with respect to water flow velocity. Peduncle length measured after five hours at each of the five experimental water velocities. Three size classes are represented: triangles represent colonies 61 to 80 mm in diameter (n = 17); circles = colonies 41 to 60 mm in diameter (n = 21); and squares = colonies 21 to 40 mm in diameter (n = 13). Mean peduncle lengths differ significantly at each experimental water velocity ($P < 0.001$; Kruskal-Wallis test). Mean of control group (33.4 mm; s.d. = 8.1) kept in still water did not differ significantly from that of the experimental group in still water ($P > 0.5$; Student's t-test). Vertical bars represent standard errors.

As a water current (directed across the dorsal surfaces of groups of 20 various-sized animals) increased in velocity from 0 to 1.2 meters per second the height-to-diameter ratio of the rachis fell. In most cases rachis diameter did not change. The response occurred within a few seconds of the increase in water velocity. A group of similar-sized control aniamls maintained in still water showed no change. Although the response was reversible, animals returned to slower water did not respond as quickly. A similar response was seen in the field. On days of little surge, rachis inflation was noticeably greater than on days of moderate surge.

The peduncle. Renilla anchors itself in the substrate with its flexible and extensible peduncle. Peduncle extension varied in a direct relation to the velocity of water flow across the rachis. Figure 4 illustrates a laboratory experiment in which animals were subjected to different current velocities. A group of various-sized sea pansies was placed initially in still water for five hours. Half of the animals were then uprooted and their rachis diameters and peduncle lengths were measured. The uprooted group was then subjected to five hour periods of various currents (0, 20, 80 and 20 cm per second). After each five hour period the rachis diameters were measured *in situ* and the peduncle lengths measured immediately upon uprooting. The other 25 of the original set of animals served as a control, maintained in still water and measured at corresponding five hour intervals. Peduncle lengths of the experimental animals were greater than those of the control animals. When returned to still water, the peduncles of the experimental animals shortened during the next five hours. Evidence that a similar process occurs under natural conditions is presented in Table I, where animals of similar size are compared for ratio of rachis diameter to peduncle length on the same day in regions subjected to different surge action (deep and shallow water) and in the same region on days of different water velocities. Rachis diameter and peduncle length were measured as in the laboratory. The results demonstrate a significant increase in peduncular lengths in the same area of bottom on days of increased surge, and among animals in the nearshore, turbulent region compared to those from deeper, quieter, water.

There are limitations to anchoring. The colony can be uprooted when strong water currents lift the edge of the rachis off the bottom and push on its surface. Since a large rachis offers a larger surface on which the water can act, a larger peduncle is necessary to withstand uprooting. While maximum peduncle extension increases with rachis diameter during growth, the ratio between peduncle length and rachis width is not constant over the size range, and small sea pansies have proportionally longer peduncles than larger colonies (Table II). As a result, smaller colonies can remain anchored in swifter water than larger ones. Field evidence to this effect is presented in Figure 5. Various-sized stained sea pansies were released in compact groups in regions of different water velocities. The animals were positioned upright on the bottom but not covered with sediment. The center of the group was marked by a stake driven into the sand. Between 30 and 60

TABLE I

Mean ratio of peduncle length to rachis diameter at various positions along depth gradient and under various surge conditions. "Calm" was when surf height was 0.4 meters; "rough" was when surf height was 1.4 meters. Samples varied in number from 60 to 150 colonies. Sample means of same size class at different surge and different depth differed significantly. Sample means of different size classes at same depth or same surge condition also differed significantly ($P < .01$; ANOVA and one-sided t-tests).

Surge condition	Depth of sample	Size class of sea pansy (in mm)		
		21–40	41–60	61–80
Calm	6.0 meters	1.36	1.08	0.83
	12.0 meters	—	0.92	0.74
Rough	6.0 meters	2.82	1.75	1.36
	12.0 meters	—	1.26	1.10

Table II

Maximum extension of peduncle in relation to diameter. Small animals can extend their peduncle proportionally more than large colonies.

Rachis diameter (mm)	Peduncle length (mm)	P/R ratio
10	28	2.80
20	71	3.55
30	77	2.57
40	83	2.08
50	87	1.74
60	89	1.48
70	96	1.37
80	94	1.17
90	96	1.06
100	94	0.94

minutes after release a polypropylene line marked in meters was swung in a circle around the stake. The distance of each animal from the stake and its shoreward-seaward position with respect to the stake were recorded. The released animals were thus monitored for the following seven days. The results show that larger colonies were lost from the shallow, swift water location while smaller individuals were not. On the other hand, in the deeper, quieter release area, similiar proportions of both large and small animals were recovered. Furthermore, laboratory experiments showed that a current strong enough to uproot large colonies did not uproot smaller ones. Two animals of different sizes were placed in an aquarium equidistant from the source of a water current of known velocity. Current velocity was steadily increased until both were uprooted. The current velocities used were similar to those in the study area. In all 40 tests the larger experimental animal was uprooted first.

The autozooids. The autozooids, buffeted by water currents and moving sediment, frequently strike both the rachidial surface and each other. This buffeting does not cause their withdrawal. Individual polyps remained extended at least an hour while the colony was subjected to either the strong, oscillating flow of wave surge or strong unidirectional currents in the laboratory. In the laboratory, although sustained water movement did not cause polyp withdrawal, a sudden increase in flow across the colony causing a sharp flexure of the rachis resulted in polyp retraction. The retraction of polyps in response to water movement was thus mediated by events affecting the rachis. Rachidial flexions by water movement in which the edge of the rachis is gently lifted off the bottom are commonly seen in the field; many of these flexures, however, are not associated with polyp withdrawal.

Colony response: anchoring. Field experiments demonstrated a characteristic behavioral pattern subsequent to uprooting and preparatory to reanchoring. The sequential events following uprooting are: (1) retraction of all expanded polyps; (2) retraction of the peduncle; (3) expulsion of the water from the colony, thus flattening the rachis (Fig. 2b); (4) settling on the substrate; (5) peduncle expansion and insertion into the substrate, and (6) re-expansion of the rachis and re-extension of the autozooids. Polyp retraction occurs within five seconds of uprooting. Peduncle retraction also begins within a few seconds but is not completed as fast. Ninety-five per cent of the colonies reached complete peduncular retraction within 30 seconds after uprooting. Although expulsion of water always follows polyp retraction, it may not begin for 20 seconds after uprooting. Expulsion, once begun, was accomplished within 10 seconds. After expulsion the rachis was very much flatter (height/diameter = 0.05 to 0.08) than any resting animal (height/diameter = 0.1 to 0.5; Fig. 2a, b). The retraction and expulsion processes occurred as the animal was buffeted along the bottom by wave surge. Only when the rachis was completely flattened and had landed "polyp-side up" on the bottom, usually in the trough between two sand ripples, did the colony escape movement by water currents and become stationary. The time between uprooting and anchoring position varied with surge strength but averaged 3 minutes over a range

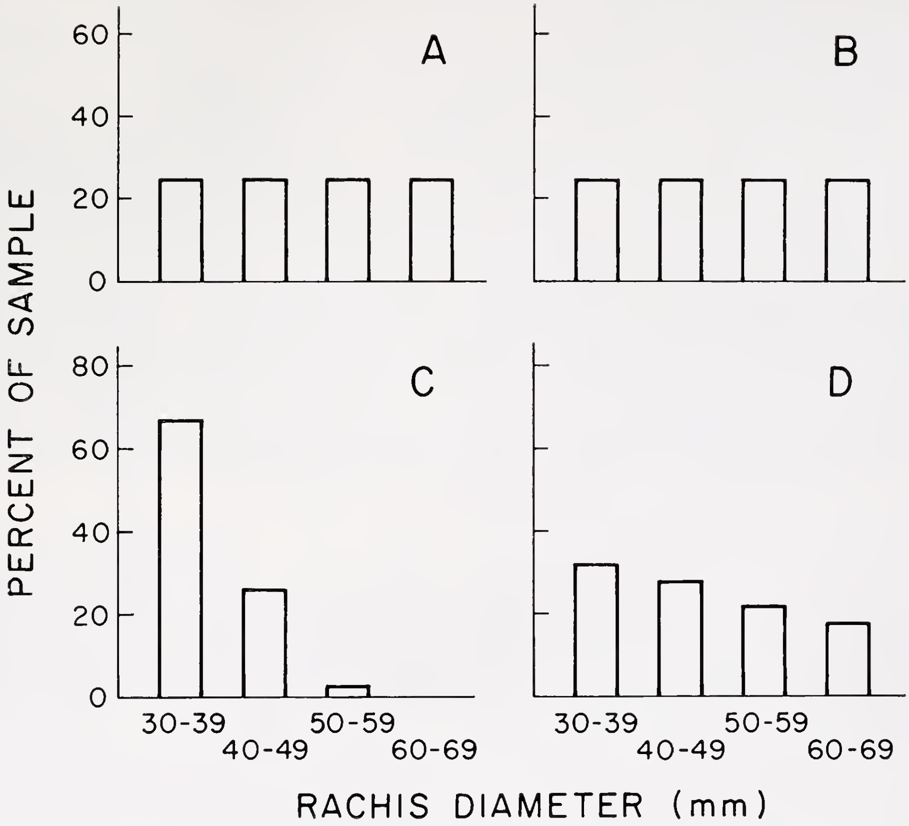


FIGURE 5. Mark-and-release experiment demonstrating differential achoring ability of various sized colonies. Initial size distributions of groups released in shallow (A; n = 100) and deep (B; n = 100) water. All animals were measured within six square meter area around release point. Size distribution of released animals one week later are given in C (shallow; n = 33) and D (deep; n = 78). The C and D distributions differ significantly ($P <$ 0.001; chi-square test), and demonstrate better anchoring ability of small colonies.

TABLE III

Laboratory emergence rates of buried R. kollikeri. *The colonies tested ranged in diameter from 35 to 45 mm.*

Amount of sand (in cm) place on experimental *R. kollikeri*	Mean time to emergence (in minutes)	Successes per 15 trials
1.0	25 ± 7	15
2.0	55 ± 16	15
3.0	92 ± 26	15
4.0	145 ± 38	12
5.0	190 ± 45	8
6.0	215 ± 62	10
7.0	240 ± 79	6

of surge conditions. Reanchoring was a lengthly process. The time to peduncle insertion varied from 15 minutes to several hours. After the peduncle had begun insertion, the rachis expanded and the polyps emerged.

Colony response: emergence. In addition to the anchoring problem, *Renilla* must deal with burial by shifting sediments. The small, constant rain of sediment normally encountered was removed by mucous strands which entangled sand grains and were then swept off the rachis by water flow and small rachidial movements. This rachidial clearing was also seen in the laboratory.

Occasionally, however, large amounts of sand are deposited on *Renilla*. Laboratory studies demonstrated that sea pansies could dig themselves out of 7.0 cm of sand. The rates of emergence varied among individuals but averaged 2.0 cm per hour (Table III). To observe the digging behavior, animals were placed in a glass cylinder of slightly larger diameter. The digging involved rachidial waves which began at the insertion of the peduncle and moved around the edge of the rachis. By flexing the rachis, sediment from above slipped below and became the surface against which the next series of waves pushed.

Interactions with predators

The sea pansy behaviors discussed so far have concerned interactions between the animal and its physical environment. The sea pansy also avoids predators behaviorally. The two principal predators of *Renilla* in southern California are the asteroid *Astropecten armatus* (a generalist feeder) and the nudibranch *Armina californica* (a pennatulid-specific feeder). *Renilla* employs different means to repel attack from these predators.

Astropecten armatus. The sea pansy fends off asteroid attack with its expanded autozooids. When the asteroid touches a polyp of a colony, it quickly lifts its arm away and often changes its direction of movement. In 200 field and laboratory observations sea pansies with expanded polyps were invulnerable to asteroid attack. However, when expanded colonies were manipulated and the polyps caused to retract, *Astropecten* crawled over the colony and began ingestion. *Astropecten* is also limited to preying upon *Renilla* of approximately 40 mm or less in diameter. Only colonies of this size have been found in the gut of the asteroid(Fig. 6). This limitation is due to *Astropecten* digesting prey within its body cavity rather than outside as many asteroids do.

Once a sea pansy was ingested by *Astropecten,* extrusion of the polyps did not effect the asteroid. The small extruded portion of the asteroid's stomach observed during ingestion showed no apparent reaction throughout 15 minutes of sustained contact with expanded polyps. However, the stomach tissue did withdraw in response to mechanical prodding and chemical (weak acid and base) stimuli.

Armina californica. *Renilla* displays a specific escape behavior in response to attack by this nudibranch. It effects escape by a specific set of sequential behaviors seen in both field and laboratory (Fig. 7): first, rapid retraction of expanded polyps; secondly, concomitant peduncular retraction; thirdly, initiation of a rachidial flexion which proceeds around the margin of the rachis; and fourthly, a characteristic fixed rachidial contraction which can be maintained for many minutes. By this behavior, the sea pansy allows itself to be uprooted from the substrate and tumbled

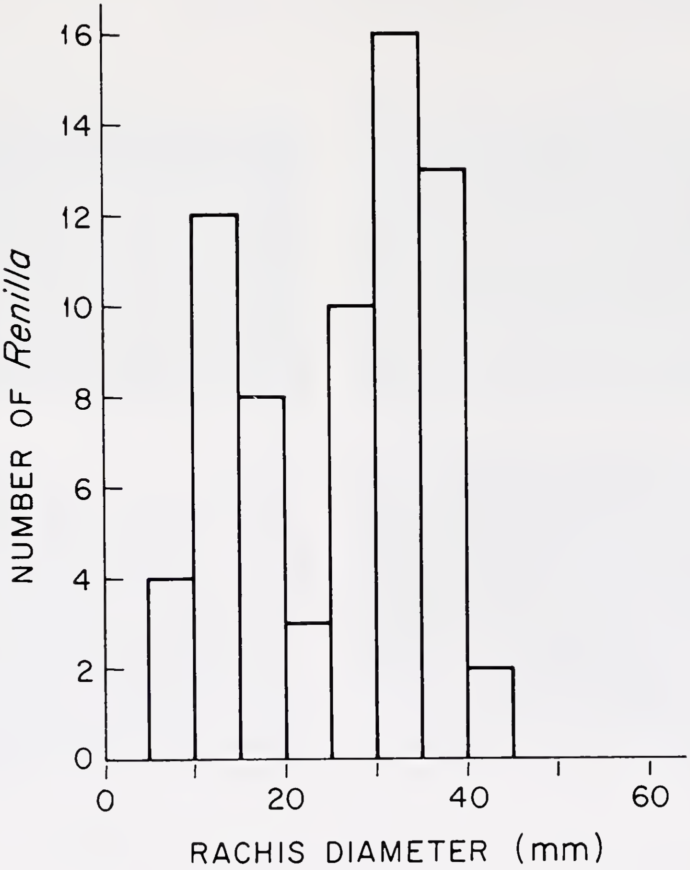


FIGURE 6. Size distribution (rachis diameter) of sea pansies ingested by *Astropecten armatus* ($n = 68$). Note that the two largest colonies were 41 mm in diameter.

away from the predator by water currents flowing along the bottom. The actual removal from the substrate usually occurred as a result of the second and third steps of the above sequence. As the edge of the rachis is lifted off the substrate, a broad area of the rachidial surface is exposed to water currents. The rachis is held turgid during these rachidial waves in a manner not seen during either the resting state or the flexions associated with sand removal. Given this stiff surface against which to act, the water uproots the animal and tumbles it along the bottom. There was considerable variation from colony to colony in response time but a general pattern was present. Within 5 to 10 seconds after attack the polyps were retracted and the peduncle began retraction. Within 20 to 40 seconds the first rachidial wave was initiated. Although the time varied with surge conditions, generally within one or two minutes after first contact with the predator the sea pansy was uprooted. On calm days with little surge, the sea pansy was sometimes not uprooted upon attack.

Figure 8 compares the distances colonies were displaced after attack by the nudibranch and physical uprooting by the experimenter. All trials were conducted on the same day under the same surge conditions. The average movement subsequent to attack was 6.6 meters (s.e. = 0.25), while that following physical

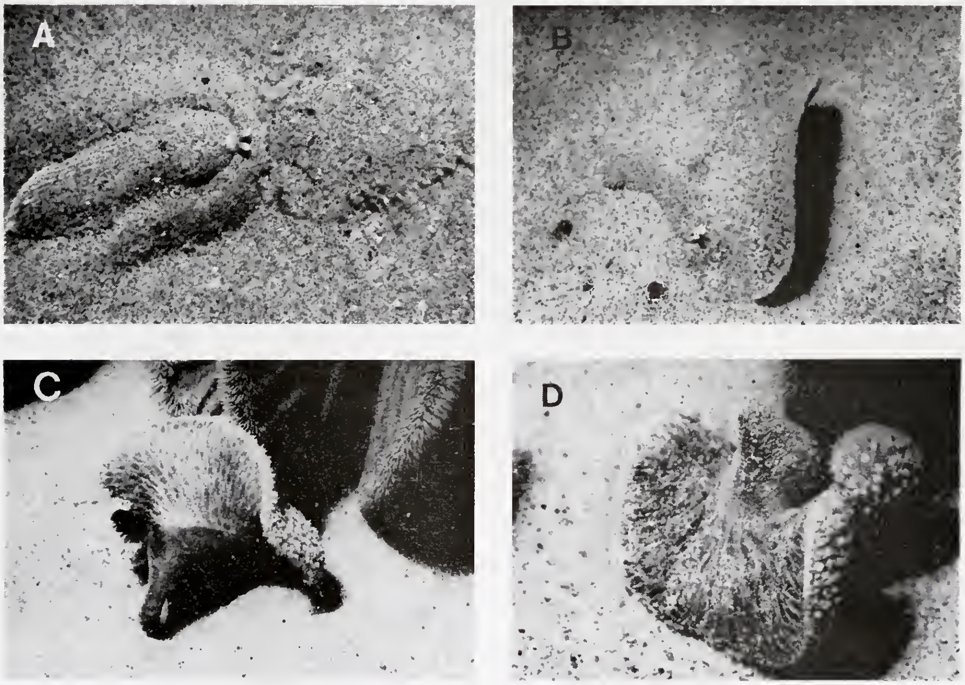


FIGURE 7. Escape behavior from *Armina californica*. A. *A. californica* approaching an expanded sea pansy. Note expanded autozooids. B. *A. californica* contacting *R. kollikeri*. Note retraction of polyps and commencement of rachidial wave. C. The sea pansy is being uprooted as rachidial waves continue and the peduncle retracts. (Animal in background is the sand dollar *Dendraster excentricus*.) D. Uprooted colony in "saddle" configuration. Note retracted peduncle. The diameter of the *Renilla* depicted is 65 mm.

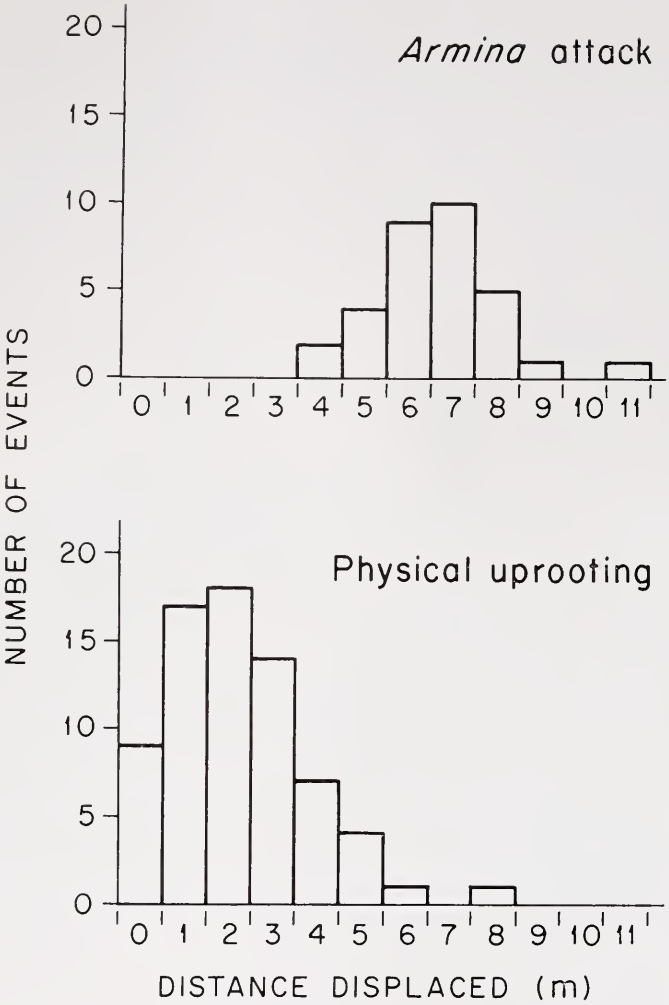


FIGURE 8. Distance between point of (A) attack by *Armina californica* ($n = 33$), and (B) uprooting by diver ($n = 60$) and point of reanchoring. The distribution means (6.6 m, s.e. $= 0.25$; and 2.3 m, s.e. $= 0.19$) differ significantly ($P < 0.001$; Student's *t*-test).

uprooting was 2.3 meters (s.e. $= 0.19$). The greater distance displaced while escaping was consistent over a wide range of surge conditions and is due to the persistence of the final rachidial contraction which may be held for several minutes. After relaxation from this configuration, the rachis and peduncle contract and the behavior preparatory to settling and reanchoring begins as described above.

It was not always necessary for the sea pansy to be tumbled away from its attacker to avoid predation. *Armina* is often swept off the colony by surge when the rachis is lifted off the bottom. However, the escape behavior continued after the nudibranch was swept away.

The effectiveness of the escape behavior is very high (Fig. 9). Over 95% of observed attacks by *Armina* ended within three minutes. Calm water, however, may thwart the sea pansy's attempt to escape. Figure 9 compares the escape efficiency on a day of moderate water movement (water velocities of about 1.2 meters per second) and on a day of quiet water (velocities of about 0.3 meters per second). Alternatively, if a sea pansy, while tumbling along the bottom, became wedged between two objects on the bottom, (*e.g.*, two sand dollars) it could not repel the nudibranch. Under these conditions colonies were seen with

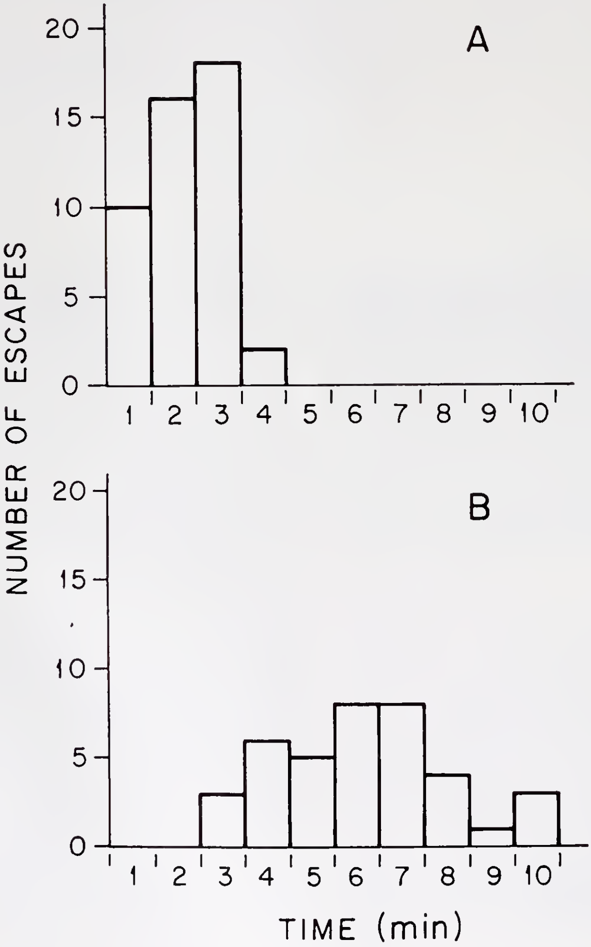


FIGURE 9. Effectiveness of escape behavior from *Armina californica*. The time to prey escape under (A) moderate surge conditions (n = 44), and under (B) calm water conditions (n = 38) is plotted. Note greater effectiveness of response with greater water movement. Means of distributions (2.3 min, s.e. = 0.12; and 6.1 min, s.e. = 0.31) differ significantly ($P <$ 0.001; Student's *t*-test).

TABLE IV

Specificity of sea pansy escape behavior. All experiments were conducted in the laboratory on anchored and expanded colonies. The "control" was a group of sea pansies in which spontaneous rachidial waves appeared during the period of the experiment.

Animal tested	Number of trials	Number of escapes in 10 min
Echinoderms		
Astropecten armatus	100	1
A. californicus	60	0
Luidia foliolata	80	2
Dendraster excentricus	100	2
Molluscs		
Armina californica	100	98
Megasurcula carpenteriana	60	5
Nassarius fossatus	60	3
Olivella biplicata	100	2
Polinices altus	75	1
Terebra pedroana	60	0
Hermissenda crassicornis	60	6
Flabillinopsis iodinea	60	4
Crustaceans		
Heterocrypta occidentalis	60	1
Randallia ornata	60	2
Cancer gracilis	100	3
"Control"	100	3

as many as six nudibranchs feeding simultaneously for periods of up to 30 minutes and were usually totally consumed.

The effectiveness of escape behavior was low for colonies smaller than 20 mm in diameter. In spite of escape movements, such colonies were small enough to be either engulfed whole or held on the bottom and consumed by the predator.

Only *Armina* elicited escape behavior of the sea pansy (Table IV). The other animals tested were errant gastropods, crustaceans, and echinoderms found within the study site.

Food and feeding

The sea pansy has difficulty capturing motile prey. In 500 encounters between polyps and 0.4 to 0.7 mm long *Artemia* nauplii, only three nauplii were caught and ingested. Similarly, in 100 encounters between polyps and copepods 0.1 mm long, no copepod was caught. However, if *Artemia* nauplii were heat-killed, *Renilla* caught and ingested them. *Renilla* could also ingest 0.1 to 0.4 mm long bits of ground mussel. The sea pansy also ingested a suspension of single-celled algae. When a colony was placed in a suspension of the alga *Dunaliella* sp., the algae were seen entangled in mucous strands on each autozooid (not on a mucous net stretching between polyps) and passed to the mouth. Within 30 minutes algae were seen within the gut, and they were not expelled within six hours after ingestion.

Discussion

The peak abundance of *Renilla kollikeri* is in shallower water than that of any other sea pen along the southern California coast. Although found in embayed areas, sea pansies are more characteristically members of the subtidal fauna of shorelines exposed to ocean swell. The strong, multidirectional water currents of this region, which greatly alter force and direction rapidly, and the resulting movement of unconsolidated substrate, greatly influence the resident fauna. The sea pansy has morphological and behavioral features which are adaptive to life in this habitat and allow it to exploit regions unsuitable to other sea pens.

Sea pansy morphology is unique among pennatulaceans with respect to a number of features. The autozooids are arrayed on a horizontally expanded rachis, in marked contrast with other sea pens (such as *Stylatula elongata* and *Virgularia* sp., which are abundant seaward and deeper than the peak abundance of *Renilla*) where they are positioned on a vertically expanded rachis. While other sea pens can extend their colonies to as much as a meter or more, the sea pansy can extend to only about 60 mm above the bottom; the maximum thickness of an expanded rachis plus the maximum height of an autozooid. The "low profile" of the sea pansy's rachis is adaptive for life in areas of strong currents since it offers minimal resistance to water flow. This low resistance is expressed by the colony's lack of orientation to water currents. Sea pens such as *Virgularia*, are oriented so that the leading, or ventral, edge of the leaf always faces into the current and turns as the current turns. The sea pansy, whose two large rachidial leaves are held against the bottom, is positioned randomly with respect to current.

In addition to these morphological features, many of the behavioral patterns of the rachis are adaptive to nearshore life. Lowering its curvature in response to increasing water flow minimizes lift forces which act to uproot the animal. Under most field conditions deflation overrides the tendency to inflate and thereby maximizes food-capturing potential. As the center of the rachis is elevated and the upper surface becomes more convex, the zooids on the central portion of the rachis are lifted higher into the water and the volume swept by the feeding zooids increases. The hemispherical feeding surface of the inflated rachis is approximately 25 per cent greater than the deflated rachis of similar diameter.

The response of *Stylatula* and *Virgularia*, which have a vertically expanded rachis, to increased surge action is to withdraw into the substrate to varying degrees. Because the feeding polyps of these sea pens are vertically arrayed, the extent of the feeding surface, and hence, the feeding ability of the colony is greatly altered by any change in the rachis extension. *Renilla*, with its horizontal array of autozooids, does not decrease the number of feeding polyps under increasing water flow.

Peduncular morphology is also adaptive for life in turbulent waters. The peduncle of the sea pansy is very extensible (see Table II) and flexible. This flexibility is afforded in part by the absence of the incompressible skeletal rod present in the peduncle of most sea pens (the genus *Renilla* is not unique in lacking a rod, and other genera, *e.g.*, *Ptilosarcus*, have reduced rods, but these conditions are highly abberant among pennatulaceans; see Kukenthal, 1915). While this greatly expanded peduncle imparts a high degree of anchoring ability, a better index of anchoring ability is the ratio of rachis height (length) to peduncle length.

Sea pens with an elongated rachis would presumably need a larger peduncle to compensate for increased water resistance. Sea pens with a large peduncle relative to the height of the rachis, would have the greatest anchoring ability. *Renilla* has the lowest rachis height/peduncle length ratio (0.08) of any genus of sea pen found in the world (measurements of the ratios of sea pens not found locally were derived from the monographs of Kukenthal, 1915; Kukenthal and Broch, 1911; Hickson, 1916).

The anchoring abilities of the sea pansy not only allow it to utilize a region of substrate not exploited by other sea pens but also determine several aspects of its own distribution. The inshore boundary differs for each size group of sea pansy within the population because larger animals cannot remain achored as well as smaller animals (Fig. 5). The differential ability of small individuals to inhabit the inshore limits of the sea pansy distribution plays an important role in the maintenance of the population as young animals inhabit areas too turbulent for effective foraging by predators. The young prey in this nearshore refuge are a source of recruitment to the offshore population of *Renilla* (Kastendiek, 1975).

In addition to maintaining its position on the bottom, the sea pansy must deal with being buried by shifting sediment. The amount of suspended sand varies with the depth of water and the strength of the wave surge. During storms the level of sediment in the shoreward limit of the sea pansy distribution can be altered by as much as 30 cm in 24 hours. *Renilla* can dig itself out of large deposits of sand suddenly placed upon it. It does so with repeated waves of rachidial flexure which sweep around the margin of the colony. Smaller deposits of sediment are cleared from the rachidial surface by the combined effects of rachidial flexures and mucous streaming. Emergence from deposits of sand and maintenance of clean surface by such mucous streaming has been reported previously among a number of corals (Yonge, 1930). One study (Marshall and Orr, 1931) suggests that the ability of a fungid coral, *Fungia* sp., to clear its upper surface of large deposits of sand enables the animal to move up through a column of sediment.

When *Renilla* is uprooted from the substrate in the field, it is generally advantageous to become re-established as quickly as possible. Prolonged buffeting may damage the colony or transport it to less favorable environments (*e.g.,* the surf zone). The most striking feature of the re-establishment behavior is the complete rachidial deflation. The degree of water expulsion from the colony is much greater under these conditions than under any other. Sand often accumulates on the surface of reanchoring animals. This layer may aid reanchoring but in the laboratory it was not necessary. The anchoring is completed with the inflation and insertion of the peduncle, which can occur while the rachis remains deflated. This ability allows anchoring to proceed while the colony is still unaffected by water movement. The process by which the peduncle digs into the substrate is similar to the process by which the sea anemones *Peachia hastata* (Ansell and Trueman, 1968) and *Phyllactis* sp. (Mangum, 1970) burrow. Peristaltic movements of a swollen region of the peduncular column travel from the distal end of the peduncle toward the rachis. Similar peristaltic movements in the rachis and peduncles of other sea pens have been described (Musgrave, 1909; Titschack, 1968).

Like the sea pansy, rod-bearing sea pens must also be stationary on the bottom to reanchor, but the morphology of these animals makes them much more susceptible to movement by water. In areas of high water velocities (greater than 2 meters per second) *Renilla* can reanchor much faster than *Stylatula*. Again, the sea pansy's low profile appears adaptive in regions of strong wave surge.

Sea pansy morphology is also influential in escape from the predatory nudibranch, *Armina californica*. Some pennatulaceans, (*e.g., Ptilosarcus guerneyi, Stylatula* and *Virgularia*) contract into the substrate when attacked. *Renilla* cannot since its flattened rachis prevents rapid withdrawal. The sea pansy avoids predation with a lateral displacement resulting from the use of the water movement it normally avoids. Upon attack the edge of the rachis is lifted and held rigid in the water current above the bottom. The sea pansy uses the rachis as a "sail" in the currents to uproot and tumble away from the attacker, a behavior quite contrary to the behavioral patterns responsible for maintenance of position on the bottom. Both the rigidity of the rachis and the "saddle" configuration (Fig. 7d) are unique to this escape response.

The escape behavior of *Renilla* is specific to *Armina* in the area studied (Table IV). Other anthozoan escape responses (for review see Ross, 1974) have been seen in rock-dwelling sea anemones and have all involved active propulsive movements. Movement in the genera *Boloceroides* and *Gonactinia* is due to synchronized tentacular sweeps and in *Stomphia* and *Actinostola* to flexions of the column. Even in still water they are capable of moving away from their original position. *Renilla*, however, depends on an external force, wave surge, for its propulsion. Its musculature affects locomotion only by positioning the animal so that the water can propel it. Because of this inability to move, the nudibranch predation on the sea pansy increases when and where surge action is weak (Kastendiek, 1975).

An interesting similarity between the *Renilla* and *Stomphia, Actinostola, Gonactinia*, (Ross, 1974), *Anthopleura* (Rosin, 1969), and *Ptilosarcus* (Birkeland, 1974) is the sensitivity of the escape behavior to nudibranch predators. As some nudibranch families have evolved specificity in feeding on coelenterates, particularly the families Eolididae and Arminidae, the cnidarian prey which represent widely divergent, taxa have convergently evolved escape behaviors.

The other major predator in the study area is the asteroid *Astropecten armatus*. This generalist predator will ingest *Renilla* smaller than about 40 mm in diameter. However the sea pansy is available as prey only when the autozooids are withdrawn. Presumably the nematocyst found in the autozooids are responsible for defending the colony from this predator.

Thus *Renilla* demonstrates two stragtegies of defense against two different predators: a behavioral escape from *Armina* and an anatomical defense against *Astropecten*. The sea pansy may use its bioluminescence as a third defense mechanism directed against a third predator type—nocturnally foraging fishes. Fish predation on the sea pansy is evidenced by teeth marks found on colonies. As nocturnal fishes have been shown to avoid a bright flash of light (Woodhead, 1966), the light emission of *Renilla* may startle fish who have nipped at the rachis. This quick deterring of the fish would account for the large number of colonies which have only a single bite taken out of them. The invertebrate predators of *Renilla*, however, are not deterred by the bioluminescence.

The feeding activity of the sea pansy is notable for both its means of prey capture and its prey selection. Like most Alcyonaria, *Renilla* is a suspension feeder. However, while an inefficient gatherer of motile animals larger than or as motile as a calanoid copepod 0.1 mm long, nonmotile prey up to the size of *Artemia* nauplii can be captured and ingested.

The sea pansy is also capable of collecting and ingesting unicellular algae. Roushdy and Hansen (1961) observed the ingestion of diatoms by another alcyonarian, *Alcyonium digitatum,* one of the few reports of phytophagy among the largely carnivorous Cnidaria. The suggestion that the sea pansy feeds on algae raises questions concerning food specialization among cnidarians. Previously, food specialization within this group was couched in terms of animal capture (*e.g.,* Yonge, 1930). However, food specialization may also be based on food type (*i.e.,* plant or animal). Increased food sources may lead to a more important role of food specialization in allowing the coexistence of many sympatric species of closely related cnidarians. The two reports of algal ingestion by cnidarians concern alcyonarians. Perhaps this group is largely restricted to feeding upon nonmotile plankton, particularly algae.

Muco-ciliary tracts on the tentacles of the polyps are important in ingesting food. Although tentacle flexures do move bits of food material into the mouth, they are not necessary. During the phytoplankton-feeding experiments a column of the single-celled algae was seen moving down the tentacle and into the mouth. The role of muco-cilary tracts in the feeding of cnidarians is known from numerous cases (*e.g.,* Yonge, 1930). MacGinitie and MacGinitie (1968) described the use of a mucous net as a feeding structure of the *Renilla* colony. During the present study such a network of mucous strands stretching from one polyp to another was observed in still water but was not used in food capture. Furthermore, a mucous net has not been observed when the colony has been in moving water. It is highly unlikely that a mucous net could persist in the nearshore areas of exposed beaches.

I gratefully acknowledge the assistance and guidance of Dr. James G. Morin during this investigation and for the field photographs. I also thank Larry Kastendiek for the laboratory photographs and Dr. Richard R. Vance, Ms. Eve Haberfield, and an anonymous reviewer for their critical readings of the manuscript.

SUMMARY

1. *Renilla kollikeri* is morphologically adapted to live in turbulent benthic areas by having a horizontally expanded rachis which offers less resistance to water flow than the vertically expanded rachis of most pennatulaceans. Furthermore, the colony is anchored by an extensible and flexible peduncle which is the largest (in relation to rachis height) among the Pennatulacea.

2. *R. kollikeri* has a number of behavioral adaptations to life in turbulent water. In response to increasing flow it alters its rachis curvature and peduncle extension to decrease resistance and increase anchoring, respectively. The sea pansy can emerge from large deposits of sediment suddenly placed upon it. Such shifts in sediment are a common occurrence in its habitat. *Renilla* has a specific

set of behavioral characteristics which re-establish the colony quickly if it is uprooted from the substrate.

3. The anchoring ability of individual colonies, which decreases as the size of the colony increases, allows small colonies to inhabit the nearshore limit of the species' distribution.

4. The sea pansy's unusual morphology contributes to its unusual behavior following attack by the nudibranch, *Armina californica.* The sea pansy positions itself so that it is uprooted from the substrate rather than withdrawing into it. By means of this species-specific behavior, the prey utilizes the prevailing water currents of its environment to avoid predation.

5. The sea pansy defends itself against different predators in different ways. It employs a behavioral escape from the specialist *Armina californica,* and an anatomical feature (presumably the nematocysts of the autozooids) and a size escape from the generalist asteroid, *Astropecten armatus.*

6. *Renilla* is restricted to preying upon largely nonmotile detrital or planktonic material. The utilization of unicellular phytoplankton is suggested.

LITERATURE CITED

ANDERSON, P. A. V., AND J. F. CASE, 1975. Electrical activity associated with luminescence and other colonial behavior in the pennatulid *Renilla kollikeri. Biol. Bull.,* **149**: 80–95.

ANSELL, A. D., AND E. E. TRUEMAN, 1968. The mechanism of burrowing in the anemone, *Peachia hastata* Gosse. *J. Exp. Mar. Biol. Ecol.,* **2**: 124–134.

BERTSCH, H., 1968. Effect of feeding by *Armina californica* on the bioluminescence of *Renilla kollikeri. Veliger,* **10**: 440–441.

BIRKELAND, C., 1974. Interactions between a sea pen and seven of its predators. *Ecol. Monogr.,* **44**: 211–232.

BUCK, J., 1973. Bioluminescent behavior in *Renilla.* I. Colonial responses. *Biol. Bull.,* **144**: 19–42.

BUISSON, B., 1971. Les activites rhythmiques comportementales de la colonie de *Veretillum cynomorium* (Cnidaria: Pennatulidae). *Cah. Biol. Mar.,* **7**: 11–48.

BUISSON, B., 1974. The behavior and the nervous system of *Veretillum cynomorium* (Cnidaria: Pennatularia). *Zool. Anz.,* **192**: 165–174.

CHAPMAN, G., 1972. A note on the oxygen consumption of *Renilla kollikeri* Pfeffer. *Comp. Biochem. Physiol.,* **42**: 863–866.

FRANCIS, L., 1973. Intraspecific aggression and its effect on the distribution of *Anthopleura elegantissima* and related sea anemones. *Biol. Bull.,* **144**: 73–92.

HICKSON, S. J., 1916. The Pennatulacea of the Siboga expedition. *Siboga Exped.,* **8(14)**: 1–256.

KASTENDIEK, J. E., 1975. The role of behavior and interspecific interactions in determining the distribution and abundance of *Renilla kollikeri* Pfeffer, a member of a subtidal sand bottom community. *Ph.D. thesis, University of California, Los Angeles,* Los Angeles, California, 194 pp.

KUKENTHAL, W., 1915. Pennatularia. *Tierreich,* **43**: 1–132.

KUKENTHAL, W., AND H. BROCH, 1911. Pennatulacea. *Wissenschftlichen Ergebnisse der Deutschen Tiefsee-expedition auf der Dampfer "Valdivia" 1898–1899,* **13**: 113–576.

LANG, J., 1971. Interspecific aggression by scleractinian corals. 1. The rediscovery of *Scolymia cubensis* (Milne Edwards & Haime). *Bull. Mar. Sci.,* **21**: 952–959.

LANG, J., 1973. Interspecific aggression by scleractinian corals. 2. Why the race is not only to the swift. *Bull. Mar. Sci.,* **23**: 260–279.

MACGINITIE, G. E., AND N. MACGINITIE, 1968. *Natural history of marine animals,* 2nd ed. McGraw-Hill, New York, 523 pp.

MACKIE, G. O., 1974. Locomotion, flotation, and dispersal. Pages 313–357 in L. Muscatine and H. Lenoff, Eds., *Coelenterate biology: reviews and new persepectives.* Academic Press, New York.

MAGNUS, D., 1966. Zur Okologie einer nachtaktiven Flachwasser-seefeder (Octocorallia: Pennatularia) im Roten Meer. *Veroeff. Inst. Meeresforsch. Bremerhaven,* **2**: 369–380.

MANGUM, D. C., 1970. Burrowing behavior of the sea anemone *Phyllactis. Biol. Bull.,* **138**: 316–325.

MARSHALL, S. M., AND A. P. ORR, 1931. Sedimentation on Low Isles Reef and its relation to coral growth. *Great Barrier Reef Expedition Sci. Rep.,* **1**: 93-133.

MORI, S., 1960. Influence of environmental and physiological factors on the daily rhythmic activity of a sea pen. *Cold Spring Harbor Symp.,* **20**: 333–344.

MUSGRAVE, E. M., 1909. Experimental observations on the organs of circulation and the powers of locomotion in Pennatulids. *Quart. J. Microsc. Sci.,* **54**: 443-481.

PARKER, C. H., 1919. The organization of *Renilla. J. Exp. Zool.,* **27**: 499–507.

PARKER, C. H., 1920a. Activities of colonial animals. I. Circulation of water in *Renilla. J. Exp. Zool.,* **31**: 343–367.

PARKER, C. H., 1920b. Activities of colonial animals. II. Neuromuscular movements and phosphorescence in *Renilla. J. Exp. Zool.,* **31**: 475–515.

ROSIN, R., 1969. Escape response of the sea anemone *Anthopleura nigrescens* to the eolid nudibranch *Herviella. Veliger,* **12**: 74–77.

ROSS, D. M., 1974. Behavior patterns in association and interactions with other animals. Pages 281–312 in L. Muscatine and H. Lenoff, Eds., *Coelenterate biology: reviews and new perspectives.* Academic Press, New York.

ROUSHDY, H. M., AND V. K. HANSEN, 1961. Filtration of phytoplankton by the octocoral *Alcyonium digitatum* L. *Nature,* **190**: 649–650.

TITSCHACK, H., 1968. Uber das nervensystem der seefeder *Veretillum cynomorium* (Pallas). *Z. Zellforsch. Mikrosk. Anat.,* **90**: 347-371.

WOODHEAD, P. M. J., 1966. The behavior of fish in relation to light in the sea. *Oceanogr. Mar. Biol. Annu. Rev.,* **4**: 337–403.

YONGE, C. M., 1930. Studies on the physiology of corals. I. Feeding mechanisms and food. *Great Barrier Reef Expedition Sci. Rep.,* **1**: 13–57.

Reference: *Biol. Bull.* **151**: 538–547. (December, 1976)

THERMAL COMPENSATION IN PROTEIN AND RNA SYNTHESIS DURING THE INTERMOLT CYCLE OF THE AMERICAN LOBSTER, *HOMARUS AMERICANUS*

J. F. McCARTHY[1], A. N. SASTRY AND G. C. TREMBLAY

Graduate School of Oceanography and Department of Biochemistry, University of Rhode Island, Kingston, Rhode Island 02881

The success of a species depends upon its ability to survive, grow and reproduce in its natural environment. The growth pattern in decapod crustaceans is known as the intermolt cycle. The survival and effective competition of most poikilothermic animals is aided by the phenomenon of thermal acclimation, enabling the organism to achieve a relative constancy of metabolic function in an environment of fluctuating temperature. Numerous reviews have delineated the myriad physiological and biochemical alterations associated with both molting and temperature acclimation (Passano, 1960; Huggins and Munday, 1968; Yamaoka and Scheer, 1970; Hohnke and Scheer, 1970; Rao, 1967; Hazel and Prosser, 1974). Given the diverse and sometimes conflicting metabolic demands associated with the two processes, it would be expected that the molt cycle condition would affect the patterns of thermal acclimation in crustaceans. McWhinnie and O'Connor (1967) reported that intermolt crayfish adaptively increase their oxygen consumption in response to cold temperatures, while premolt animals do not compensate.

The purpose of the present study is to explore the effect of the internal physiological condition of the organism (molt cycle stage) upon the metabolic response to an imposed environmental stress (acclimation temperature). The rates of incorporation of precursors into protein and RNA were measured to provide both specific information on changes in two important pathways, and more general information on metabolic changes because of the central role of these pathways in the synthesis of new enzymes and cellular components. Since pyrimidine precursors may enter the nucleotide pool for subsequent incorporation into RNA through a salvage pathway (uridine → UMP → RNA) or through *de novo* synthesis ($CO_2 \rightarrow$ orotic acid $\rightarrow$ UMP $\rightarrow$ RNA), the activities of both pathways were monitored.

MATERIALS AND METHODS

Animals

Lobsters were collected from Narragansett Bay, Rhode Island, between late May and early September. Only reproductively immature animals, with a carapace length of 55–70 mm, were used. Molt condition was determined by the pleopod setogenesis method of Aiken (1973). Lobsters were acclimated at least one month in Dayno recirculating temperature-controlled aquaria, and fed three times weekly on chopped fish and mussels.

---

[1] Present address: Biology Division, Oak Ridge National Laboratory, P. O. Box Y, Oak Ridge, Tennessee 37830.

In vitro *assays*

The rate of protein synthesis and the activity of the salvage pathway for pyrimidine biosynthesis were assessed by measuring *in vitro* the rate of incorporation of ^{3}H-leucine or ^{3}H-uridine into the acid-insoluble fraction. The activity of the *de novo* pyrimidine pathway was assessed in two steps: the rate of incorporation of $NaH^{14}CO_3$ into orotic acid, and the rate of incorporation of orotic-^{14}C-acid into the acid-insoluble fraction. In all experiments, minces of 250 mg of tissue were incubated in 10 ml of lobster physiological saline (Cole, 1941) with 20 mM glucose and 30 mM imidazole buffer (pH 7.6) and adjusted to a saturating concentration of radioactive precursor [50 mM in ^{3}H-leucine (5 μCi); 20 mM in ^{3}H-uridine (5 μCi); 1 mM in orotic-^{14}C-acid (1 μCi)]; or 15 mM in $NaH^{14}CO_3$ (30 μCi), and 10 mM in 6-azauridine which inhibits the further metabolism of orotic acid (Handschumacher and Pasternak, 1958). All tissues showed linear incorporation with time for at least two hours. Saturating concentrations of nonradioactive precursor were added to the incubation medium to minimize variations in uptake and incorporation of the radioisotope due to alterations in membrane permeability and endogeneous precursor pool size. The saturation levels were determined by increasing the concentration of nonradioactive precursor while maintaining a constant specific activity (μCi/mmole) until there was no further enhancement of the incorporation rate.

After two hours of incubation with shaking at the desired temperature, the reaction was terminated with 10 ml ice cold 1 N $HC10_4$. The acid-insoluble, lipid-free residue was prepared by homogenizing the incubation mixture and washing the precipitate four times in 10 ml of 0.5 N $HC10_4$, once in water, and successively in 10 ml each of ethanol, ethanol/ether (1 : 1), and ethyl ether. The residue was dried, weighed and solubilized with 0.5 ml of Soluene-100 (Packard Instrument Corp.). Scintillation fluid (Das, 1967) was added and the samples were counted on a Packard Tri-Carb Model 3033 or Beckman Model 150 liquid scintillation counter for the time required to give a standard deviation of no greater than 5% of the total activity. Counting efficiency was about 30% for tritium and 70% for carbon-14.

The orotic acid synthesized during incubation with $NaH^{14}CO_3$ was isolated by co-crystalization with carrier orotate (Smith *et al.*, 1973), and recrystalized to a constant specific activity. The crystals were dissolved in 0.25 N KOH, diluted with Aquasol LSC cocktail (Packard Instruments) and counted in a Beckman Model 150 liquid scintillation counter for the time required to give a maximum standard deviation of 1.5% of the total activity.

RNA content

The RNA content of the tissues was determined by the orcinal colorimetric method of Drury (1948) performed on the acidified supernatant resulting from a mild alkaline hydrolysis of the acid-insoluble, lipid-free residue (0.3 N KOH for 2 hours at 37° C). Between 98–99% of the ^{3}H-uridine contained in the acid-

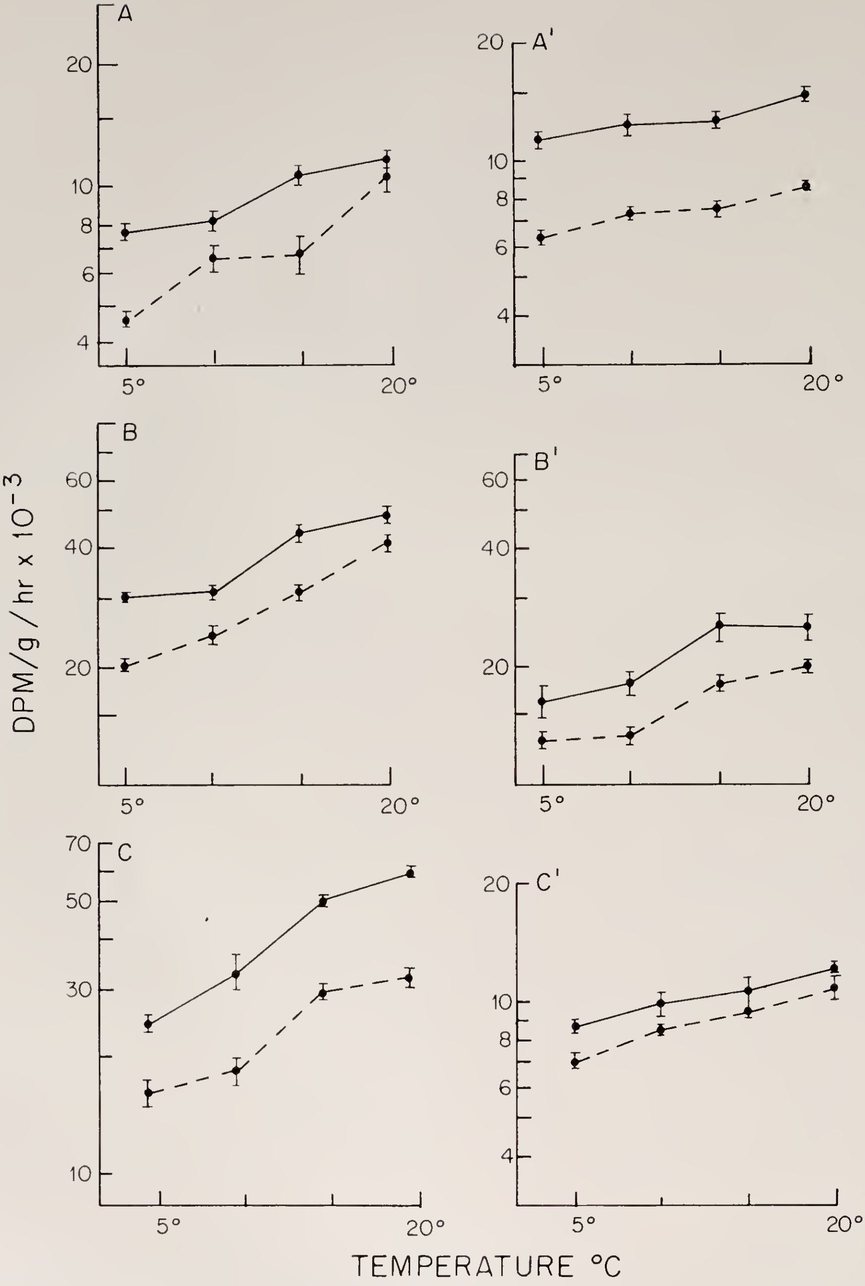


FIGURE 1. *Intermolt lobsters:* the effect of temperature acclimation on the rate of incorporation of ^{3}H-leucine (A, B, C) and ^{3}H-uridine (A′, B′, C′) into the acid insoluble fraction of midgut gland (A, A′), abdominal muscle (B, B′) and gill (C, C′). Solid lines are data from 5° C-acclimated lobsters, broken lines are data from 20° C-acclimated lobsters. Vertical

insoluble residue was rendered acid-soluble by mild alkaline hydrolysis, providing evidence that virtually all of the incorporation into nucleic acids is into RNA.

Results

The effect of the molt cycle stage upon the capacity for thermal acclimation in lobster tissues is shown in Figures 1 and 2. In these experiments, intermolt (stage C_4) (Fig. 1) and premolt (stage D_1'''-D_2) (Fig. 2) lobsters were acclimated to 5° C or 20° C for one month before tissues were assayed *in vitro* at a variety of temperatures. Temperature acclimation of intermolt lobsters results in compensatory shifts in the rates of incorporation of both ^{3}H-leucine and ^{3}H-uridine. In midgut gland, abdominal muscle, and gill tissue, the rate-versus-temperature (R-T) curve for tissue from cold-acclimated lobsters is translated to the left of that of warm-adapted animals.

In contrast, midgut gland and muscle from acclimated premolt lobsters (Figure 2) show no thermal compensation or an inverse compensation. Incorporation rates of warm-adapted tissue are either identical with or greater than incorporation rates of cold-adapted tissue.

Unlike the other premolt tissues, incorporation rates in gill exhibit a rotation, or change in the slope, of the R-T curves. Cold-acclimated curves are rotated counterclockwise with respect to leucine incorporation and slightly clockwise with respect to uridine incorporation.

The possibility of a difference in the rate of acclimation between intermolt and premolt animals was tested. Animals acclimated to one temperature regime were transferred to the other temperature; the rates of leucine and uridine incorporation were assayed every 3–4 days for a month. Most of the alterations in the elevation and slope of the R-T curves for the midgut gland and muscles of both intermolt and premolt lobsters were accomplished within ten days of transfer to the new temperature. The one exception was in the uridine incorporation rates in the intermolt midgut gland. The rates remained at warm-acclimated levels after ten days at 5° C, even though ten days was sufficient time for an adaptive response in the reciprocal acclimation direction (McCarthy, 1974).

The results of the studies on the activity of the *de novo* pathway for pyrimidine biosynthesis are shown in Table I. The rate of incorporation of $NaH^{14}CO_3$ into orotic acid in tissue of intermolt lobsters is relatively independent of the acclimation temperature. Alteration of the thermal regime has a more pronounced effect in tissues of premolt animals. Warm-acclimated rates are 25–30% higher than cold-adapted rates (inverse compensation). In 20° C-adapted lobsters, incorporation of $NaH^{14}CO_3$ into orotic acid in premolt tissue is 17–24% greater than in intermolt tissue.

The rate of incorporation of orotic-6-^{14}C acid into RNA is greater in cold-acclimated lobsters of either molt cycle condition, suggesting a compensatory acclimation to temperature. The activity of this portion of the pathway in the midgut gland of premolt lobsters is twice that of intermolt animals; activity is slightly

---

bars indicate ± one standard error. Under experimental conditions, 1×10^3 DPM per gram tissue per hr is equivalent to the conversion of 45.0 nanomoles of ^{3}H-leucine into protein or 18.2 nanomoles of ^{3}H-uridine into RNA.

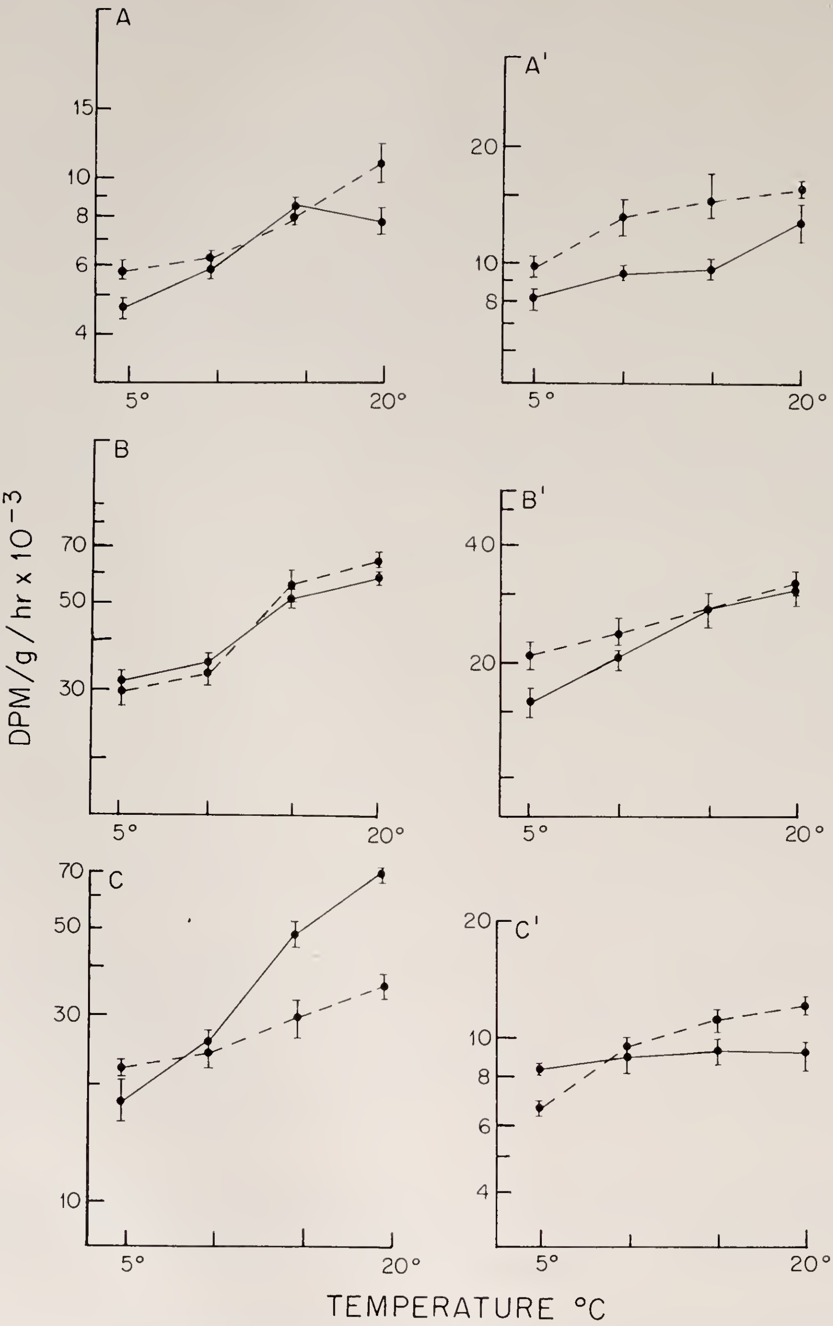


FIGURE 2. *Premolt lobsters:* the data are presented in the same way as in Figure 1.

greater in the muscle of intermolt as compared to premolt lobsters. There is a consistent decrease in the rate of orotate incorporation at increased incubation temperatures. The cause of this decrease is not immediately apparent; however, it is not due to a decreased rate of conversion of orotic acid to UMP. The rate of decarboxylation of orotic-2-^{14}C acid measured at 20° C was found to be 2–3 times greater than the rate at 5° C.

Both the molt cycle condition and temperature regime alter the concentration of RNA in the tissues (Table I). The RNA content of premolt mid-gut gland is 30–40% greater than that of intermolt tissue. The molt cycle had less effect upon the RNA content of the muscle and gill (McCarthy, 1974). Cold acclimation of both intermolt and premolt lobsters resulted in an increase in the RNA content of muscle and gill, but a decrease in hepatopancreas RNA.

Discussion

The response of an organism to an environmental stress such as temperature is clearly influenced by the internal physiological state of the animal. In this study, intermolt lobsters exhibited a positive acclimation response with respect to the *in vitro* rate of incorporation of precursors into protein and RNA. Lobsters in mid-premolt (stage D_1'''-D_2) showed either no acclimation or inverse acclimation, except in gill tissue. These results point out the need to consider the changing physiological conditions of an organism when evaluating its capacity to adapt to proposed environmental modifications.

The differential acclimation abilities of premolt and intermolt lobsters is also reflected in their capacity for resistance acclimations. Premolt lobsters acclimated to 15° C survive exposure to 28° C for a much shorter time than do intermolt animals with the same acclimation history. Resistance to low salinity also decreases in premolt lobsters (McLeese, 1956). The capacity of intermolt lobsters for temperature acclimation is undoubtedly of adaptive advantage in the natural environment. After summer molting, the lobsters continue to feed actively during the fall and early winter, even while temperatures are decreasing. Feeding activity (as estimated by stomach fullness) begins to increase from mid-winter, before water temperatures rise significantly (Ennis, 1973). The ability to metabolically compensate for the decreased environmental temperature permits the lobster to continue its active search for food. Because of this continued feeding activity, serum protein concentrations, which drop significantly following summer molting, recover to intermolt levels by mid-winter (Ennis, 1973).

While the acclimation capacity of intermolt lobsters is clearly of adaptive advantage, the absence of this temperature acclimation in the premolt animal is less readily understood. Yet this lack of an acclimation response may be of adaptive advantage, or at least impose no disadvantage, on the premolt lobster. Having ceased feeding near the beginning of the premolt state (Weiss, 1970), the lobster must complete preparations for the molt, including the synthesis of a new cuticle, using stored organic reserves. Metabolic compensation to temperature could compete with the molting process for the utilization of these reserves. In the fiddler crab, *Uca pugnax,* depletion of available organic reserves by starvation results in a loss of the ability to respond to cold exposure by a compensatory increase in metabolic rate (Vernberg, 1959). These observations suggest that there exists

TABLE I

Pyrimidine biosynthesis in the midgut gland and abdominal muscle of lobsters of different intermolt cycle and acclimation conditions. RNA is mg/g tissue, all others in nanomoles incorporated/g tissue/hr ± 1 standard error. Number of animals is in parentheses.

Molt stage	Acclimation temperature	$NaH^{14}CO_3$		Orotic-^{14}C-acid		^{3}H-uridine		RNA
		Incubation temperature						
		5° C	20° C	5° C	20° C	5° C	20° C	
Midgut gland								
Intermolt (stage C_4)	5° C	1.64 ±0.01 (3)	1.70 ±0.03 (3)	9.73 ±1.3 (3)	7.20 ±1.0 (3)	206.2 ±10.9 (12)	266.1 ±14.6 (10)	11.6 ±0.4 (10)
	20° C	1.64 ±0.05 (2)	1.97 ±0.06 (3)	8.03 ±1.0 (5)	4.40 ±0.4 (5)	115.6 ±5.5 (7)	157.5 ±3.6 (6)	14.4 ±0.3 (11)
Premolt (stage $D_1''' - D_2$)	5° C	1.59 ±0.04 (4)	1.46 ±0.03 (3)	20.84 ±0.8 (5)	17.65 ±2.0 (5)	148.3 ±9.1 (5)	236.0 ±27.3 (5)	18.6 ±0.6 (10)
	20° C	1.98 ±0.03 (4)	2.56 ±0.08 (2)	15.40 ±0.8 (4)	8.63 ±0.4 (3)	178.0 ±12.7 (6)	284.3 ±14.6 (7)	20.7 ±1.0 (6)
Muscle								
Intermolt (stage C_4)	5° C	1.63 ±0.02 (3)	1.56 ±0.01 (3)	13.86 ±1.0 (3)	10.01 ±1.2 (3)	292.6 ±25.5 (10)	466.5 ±38.2 (9)	0.92 ±0.05 (11)
	20° C	1.61 ±0.03 (3)	1.81 ±0.02 (3)	10.17 ±0.7 (5)	6.32 ±0.5 (5)	235.7 ±10.9 (7)	364.9 ±12.7 (6)	0.86 ±0.02 (8)
Premolt (stage $D_1''' - D_2$)	5° C	1.52 ±0.04 (4)	1.70 ±0.05 (4)	11.16 ±0.9 (5)	8.08 ±0.4 (5)	286.5 ±23.7 (6)	553.3 ±58.2 (6)	0.92 ±0.03 (8)
	20° C	2.12 ±0.04 (4)	2.33 ±0.01 (3)	9.62 ±1.1 (3)	6.65 ±0.7 (3)	376.7 ±32.8(5)	572.7 ±36.4 (6)	0.72 ±0.03 (8)

an integrative mechanism capable of preventing temperature acclimation when available organic reserves are required for what appear to be more vital metabolic needs. The compensatory mechanisms may also be of less value to the premolt lobster since in temperate latitudes, molting is a seasonal occurrence cued at least in part by an increased ambient temperature (Passano, 1960). Thus, the premolt animal should infrequently encounter the prolonged low temperatures which necessitate a compensatory metabolic reorganization.

The predominant pattern of acclimation in intermolt lobster tissue is a translation of the R-T curves. This is similar to the patterns exhibited by goldfish tissue with respect to leucine incorporation (Das and Prosser, 1967). It is also consistent with the mechanism for increased protein synthesis proposed by Haschemeyer (1968, 1969a, b), who found that the activity of elongation factor I in the protein synthetic pathway increased upon cold acclimation, resulting in a nonspecific increase in the rate of addition of aminoacyl residues to growing polypeptide chains. Such an increased rate of substrate addition would be expected to result in a translation, *i.e.,* a change in the elevation, but not the slope, of the R-T curves (Prosser and Brown, 1962). In this study, the cold-induced increase in the rate of leucine incorporation in intermolt midgut gland occurred several days before the corresponding increase in the rate of uridine incorporation or in the total RNA content of the tissue (McCarthy, 1974). This is also consistent with such a non-RNA mediated increase in the rate of protein synthesis.

While the molt cycle clearly affects the rates of incorporation of precursors into protein and RNA, the direction and magnitude of the change varies with the acclimation condition, incubation temperature, and tissue. Skinner (1965, 1968) and Gorell and Gilbert (1971) found an increase in both the level of leucine incorporation into proteins and the RNA content of the tissues of premolt animals and attempted to interpret the correlation as causative. The present study does not find a uniform increase in the rate of leucine incorporation. Yamaoka (1972) also found arginine incorporation in the crab, *Cancer magister,* to be greater in intermolt than premolt tissues. It seems likely, then, that control of the rate of protein synthesis during the molt cycle is not directly dependent upon the level of RNA in the tissues.

Although Skinner (1966) in *Gecarcinus lateralis* and Gorell and Gilbert (1971) in *Orconectes virilis* found RNA levels to increase in the premolt midgut gland, they reported a decrease and no change, respectively, in the rate of ^{3}H-uridine incorporation into RNA. The present study reports over a 50% increase in the rate of uridine incorporation in warm-adapted premolt midgut gland and abdominal muscle. In an effort to resolve this discrepancy, the possibility of competition between the salvage and *de novo* pathways for pyrimidine biosynthesis was examined. By comparison of premolt and intermolt rates measured in 20° C-acclimated lobsters assayed at 20° C (the more "normal" environmental conditions for premolt lobsters), it is clear that in both portions of the *de novo* pathway, as well as in the salvage pathway, there is an increase in amount of precursor (in nanomoles) incorporated into RNA in the premolt stage. This increased synthesis of pyrimidine nucleotides from both pathways is clearly the source of nucleotides for the increased level of RNA in premolt tissue.

The causes for the decrease in the conversion of orotate to RNA at increasing incubation temperatures is not fully elucidated. However, it is not due to a decreased rate of conversion of orotic acid to UMP, which was shown to increase at the higher incubation temperature. It is possible that the decreased incorporation into RNA is due to an increase in competition for nucleotides for purposes other than nucleic acid synthesis. The increased level of chitin biosynthesis beginning at stage D_1 may account for the increased competition for the pyrimidine nucleotides, The rate of ^{14}C-acetylglucosamine incorporation into both chitin and the activated intermediate, UDP-acetylglucosamine, increases by several orders of magnitude beginning at stage D_1 (Gwinn and Stevenson, 1973a, b). Although the nucleotide is recycled during the synthesis of the polysaccharide, the dramatically increased rate of chitin formation would presumably divert some of the pyrimidine nucleotides from nucleic acid metabolism.

Such an increase in competition for nucleotides would account for the decreased incorporation of ^{3}H-uridine into RNA found by the other workers. This effect would be minimized in the system used in this study, which measured incorporation in the presence of a saturating concentration of precursor. It should be noted that the amount of uridine incorporated into RNA (in nanomoles) by this system appears to be very much greater than that accounted for by increased competition for nucleotides at elevated incubation temperatures. It is unlikely, therefore, that the R-T curves for uridine incorporation are significantly influenced by this effect.

The incorporation of $NaH^{14}CO_3$ into orotic acid and orotic-^{14}C-acid into RNA reported here is, to our knowledge, the first report which demonstrates the existence of the complete *de novo* pathway of pyrimidine biosynthesis in the class Crustacea. Previously the pathway has been reported in the class Insecta (Porembska, Gorzkowski, and Jezewska, 1966; Moriuchi, Koga, Yamada, and Akune, 1972).

This work was supported by the office of Sea Grant Programs, NOAA, and based on a Ph.D. thesis (by J. F. McCarthy) submitted to the University of Rhode Island. We thank Dr. E. Jackim for his advice during this study and Dr. D. M. Skinner for her critical reading of the manuscript.

SUMMARY

1. The *in vitro* rates of incorporation of precursors into protein and RNA and the concentration of RNA were measured in tissues of intermolt and premolt lobsters acclimated to 5° C and 20° C. Midgut gland, abdominal muscle and gill of intermolt lobsters respond to temperature acclimation by a compensatory translation of the rate-temperature (R-T) curves with respect to the rates of incorporation of ^{3}H-leucine and ^{3}H-uridine into the acid-insoluble fraction. Midgut gland and muscle of premolt animals exhibit either no compensation or inverse compensation; gill tissue exhibits a rotation of the R-T curve.

2. The existence of the complete *de novo* pathway of pyrimidine biosynthesis is demonstrated in the class Crustacea. $NaH^{14}CO_2$ is incorporated into orotic acid and orotic-^{14}C-acid is incorporated into the acid-insoluble fraction.

3. Both the concentration of RNA and the rates of incorporation of precursors of both the salvage and *de novo* pyrimidine pathways are enhanced in the midgut gland of premolt lobsters, relative to intermolt tissue, under conditions of warm-acclimation.

LITERATURE CITED

Aiken, D. E., 1973. Proecdysis, setal development, and molt prediction in the American lobster (*Homarus americanus*). *J. Fish. Res. Board Can.*, **30**: 1337–1344.

Cole, W. H., 1941. A perfusing solution for the lobster (*Homarus*) heart and the effects of its constituent ions on the heart. *J. Gen. Physiol.*, **25**: 1–6.

Das, A. B., 1967. Biochemical changes in tissues of goldfish acclimated to high and low temperature—II. Synthesis of protein and RNA of subcellular fractions and tissue composition. *Comp. Biochem. Physiol.*, **21**: 469–485.

Das, A. B., and C. L. Prosser, 1967. Biochemical changes in tissues of goldfish acclimated to high and low temperature—I. Protein synthesis. *Comp. Biochem. Physiol.*, **21**: 449–467.

Drury, H. F., 1948. Identification and estimation of pentoses in the presence of glucose. *Arch. Biochem.*, **19**: 455–466.

Ennis, G. P., 1973. Food, feeding, and condition of lobsters, *Homarus americanus*, throughout the seasonal cycle in Bonavista Bay, Newfoundland. *J. Fish. Res. Board Can.*, **30**: 1905–1909.

Gorell, T. A. and L. I. Gilbert, 1971. Protein and RNA synthesis in the premolt crayfish, *Orconectes virilis*. *Z. Vergl. Physiol.*, **73**: 345–356.

Gwinn, J. F. and J. R. Stevenson, 1973a. Role of acetylglucosamine in chitin synthesis in crayfish. I. Correlation of ^{14}C-acetylglucosamine incorporated with stages of the molting cycle. *Comp. Biochem. Physiol.*, **45B**: 769–776.

Gwinn, J. F. and J. R. Stevenson, 1973b. Role of acetylglucosamine in chitin synthesis in crayfiish. II. Enzymes in the epidermis for incorporation of acetylglucosamine into UDP-acetylglucosamine. *Comp. Biochem. Physiol.*, **45B**: 777–785.

Handschumacher, R. E. and C. A. Pasternak, 1958. Inhibition of orotidylic acid decarboxylase, a primary site of carcinostasis by 6-azauracil. *Biochim. Biophys. Acta*, **30**: 451–452.

Haschemeyer, A. E. V., 1968. Compensation of liver protein synthesis in temperature-acclimated toadfish, *Opsanus tau*. *Biol. Bull.*, **135**: 130–140.

Haschemeyer, A. E. V., 1969a. Studies on the control of protein synthesis in low temperature acclimation. *Comp. Biochem. Physiol.,* **28**: 535–552.

Haschemeyer, A. E. V., 1969b. Rates of polypeptide chain assembly in liver *in vivo:* Relation to the mechanism of temperature acclimation in *Opsanus tau. Proc. Nat. Acad. Sci. U.S.A.,* **62**: 128–135.

Hazel, J. R. and C. L. Prosser, 1974. Molecular mechanisms of temperature compensation in poikilotherms. *Physiol. Rev.,* **54**: 620–677.

Hohnke, L. and B. T. Scheer, 1970. Carbohydrate metabolism in Crustaceans. Pages 147–166 in M. Florkin and B. T. Scheer, Eds., *Chemical Zoology, V, A. Crustaceans.* Academic Press, New York.

Huggins, A. K. and K. A. Munday, 1968. Crustacean metabolism. *Adv. Comp. Physiol. Biochem.,* **3**: 271–378.

McCarthy, J. F., 1975. Temperature compensation in protein and RNA synthesis during the intermolt cycle of the American lobster, *Homarus americanus.* Ph.D. Thesis, University of Rhode Island, Kingston, Rhode Island, 140 pp. (*Diss. Abstr.,* **36B**: 610B; order no. 75–17,866.)

McLeese, D. W., 1956. Effects of temperature, salinity and oxygen on the survival of the American lobster. *J. Fish. Res. Board Can.,* **13**: 247–272.

McWhinnie, M., and J. D. O'Connor, 1967. Metabolism and low temperature acclimation in the temperate crayfish, *Orconectes virlis. Comp. Biochem. Physiol.,* **20**: 131–145.

Moriuchi, A., K. Koga, J. Yamada, and S. Akune, 1972. DNA synthesis and activities of pyrimidine synthesizing enzymes in the silk gland of *Bombyx mori. J. Insect. Physiol.,* **18**: 1463–1476.

Passano, L. M., 1960. Molting and its control. Pages 473–536 in T. H. Waterman, Ed., *The physiology of Crustacea. I. Metabolism and growth.* Academic Press, New York.

Porembska, A., B. Gorzkowski, and M. Jezewska, 1966. Utilization of 6-^{14}C orotate in the biosynthesis of pyrimidines in *Helix pomatia* and *Celerio euphorbiae. Acta Biochim. Polonica,* **13**: 107–111.

Prosser, C. L. and F. A. Brown, 1962. *Comparative animal physiology.* W. B. Saunders Co., Philadelphia, Pennsylvania, 688 pp.

Rao, K. P., 1967. Biochemical correlates of temperature acclimation. Pages 227–244 in C. L. Prosser, Ed., *Molecular mechanisms of temperature adaptation.* AAAS Publ. No. 84, Washington, D. C.

Skinner, D. M., 1965. Amino acid incorporation into protein during the molt cycle of the land crab, *Gecarcinus lateralis. J. Exp. Zool.,* **160**: 225–234.

Skinner, D. M., 1966. Macromolecular changes associated with the growth of crustacean tissues. *Amer. Zool.,* **6**: 235–242.

Skinner, D. M., 1968. Isolation and characterization of ribosomal ribonucleic acid from the crustacean, *Gecarcinus lateralis. J. Exp. Zool.,* **169**: 347–356.

Smith, P. C., C. E. Knott, and G. C. Tremblay, 1973. Detection of the feedback control of pyrimidine biosynthesis in slices of several rat tissues. *Biochem. Biophys. Res. Commun.,* **55**: 1141–1146.

Vernberg, F. J., 1959. Studies on the physiological variation between tropical and temperate zone fiddler crabs of the genus *Uca.* III. The influence of temperature acclimation on oxygen consumption of whole organisms. *Biol. Bull.,* **117**: 582–593.

Weiss, H. M., 1970. The diet and feeding behavior of the lobster, *Homarus americanus* in Long Island Sound. *Ph.D. Thesis, University of Connecticut,* Storrs, Connecticut, 104 pp. (*Diss. Abstr.,* **31B**: 7245-B; order no. 71-16, 057.)

Yamaoka, L. H., 1972. Protein synthesis in decapod crustaceans: The incorporation of ^{14}C-L-arginine into protein during the intermolt cycle in the dungeness crab, *Cancer magister. Ph.D. Thesis, University of Oregon,* Eugene, Oregon, 114 pp. (*Diss. Abstr.,* **33B**: 4481–B; order no. 73–7981.)

Yamaoka, L. H. and B. T. Scheer, 1970. Chemistry of growth and development. Pages 321–342 in M. Florkin and B. T. Scheer, Eds., *Chemical zoology, V, A. Crustaceans.* Academic Press, New York.

Reference: *Biol. Bull.* **151**: 548-559. (December, 1976)

MORPHOLOGICAL ADAPTATION TO THERMAL STRESS IN A MARINE FISH, *FUNDULUS HETEROCLITUS*[1, 2]

JEFFRY B. MITTON[3] AND RICHARD K. KOEHN

Department of Ecology and Evolution, State University of New York at Stony Brook, Stony Brook, New York 11790

Clinal morphological variation of marine fishes has been observed within and across species (Barlow, 1961). This variation is generally associated with differences in the thermal regime and can be due primarily to various developmental responses to environmental cues (Fowler, 1970) or to frequency differences of polymorphic genes. In either case, once latitudinal patterns of variation have been described, temperature stresses for a particular population may be estimated. The effects of a thermal environmental perturbation upon the morphological characteristics of a population of *Fundulus heteroclitus* (Pisces: Cyprinodontidae) and an interpretation of how these effects may be of adaptive significance to natural populations experiencing relatively high temperature are presented in this study.

MATERIALS AND METHODS

Fundulus heteroclitus is a common fish in salt and brackish water from Newfoundland to the Mantanzas River in northern Florida. Latitudinal variation is characterized here with population samples in Long Island Sound, and Ladies Island, South Carolina. Two control populations in Long Island Sound flank a thermal effluent produced by an electric generating plant at Northport, New York, on the north shore of Long Island. One control locality is a large salt marsh approximately fifteen miles east of Northport (Flax Pond), and another is a harbor five miles west of Northport (Centerport). Temperatures during this study were 12° to 15° C higher at Northport than at either flanking control locality, which varied from 0° C in winter to about 20° C in summer. Summer temperatures at Northport approached the thermal tolerance limit of *F. heteroclitus*. Maximum temperatures at Ladies Island, South Carolina, are 3° to 5° C warmer than those in the control localities in Long Island Sound, and the mean temperature at the southern locality is 5° to 7° C higher than the northern control localities. Specimens were collected in traps (cylindrical wire cages with conical indentations at each end) baited with crushed mussels.

For comparison of population samples, the following characters were recorded for each fish: sex, standard length, predorsal length, head length, snout length,

---

[1] Contribution No. 144 from the Program in Ecology and Evolution, State University of New York, Stony Brook, New York 11790.

[2] Taken from a dissertation submitted in partial requirement for the degree of Doctor of Philosophy at the State University of New York.

[3] Present address: Department of Environmental, Population, and Organismic Biology and Institute for Behavioral Genetics, University of Colorado, Boulder, Colorado 80309.

interorbital width, length of dorsal fin insertion, peduncle length, peduncle width, lateral line scales, circumpeduncular scales, predorsal scales, scales above the lateral line, scales below the lateral line, pectoral fin rays, dorsal fin rays, caudal fin rays, and anal fin rays. Morphological measurements followed the criteria used by Hubbs and Lagler (1949), with the following exceptions. Dorsal and anal fin ray counts include all rays that were separated at the base. Caudal fin rays include only the branched rays. Lateral line scales start with the most anterior scale that is fully developed and pored.

The mean standard lengths of specimens from different localities are heterogeneous. In an effort to reduce this heterogeneity, length and width measurements were divided by the standard length of the specimen, and the variable "standard length" was dropped from consideration. Gould (1966, 1971) and Atchley, Gaskins, and Anderson (1976) have shown that this procedure does not necessarily remove all of the size effects and will introduce spurious correlations between standardized variables. The standardization of length and width measurements will remove the size effect for isometric measurements and has been used here for lack of a better technique that may be used upon the available data. The distortion of the covariance structure introduced by standardization is heavily dependent upon the range of relative magnitudes of the coefficients of variation of the numerator and denominator variables (Atchley, *et al.*, 1976). Although the variances of the standard lengths are heteroscedastic, the range of the coefficients of variation (9.6–15.8) is not great. The standardization will alter the correlations

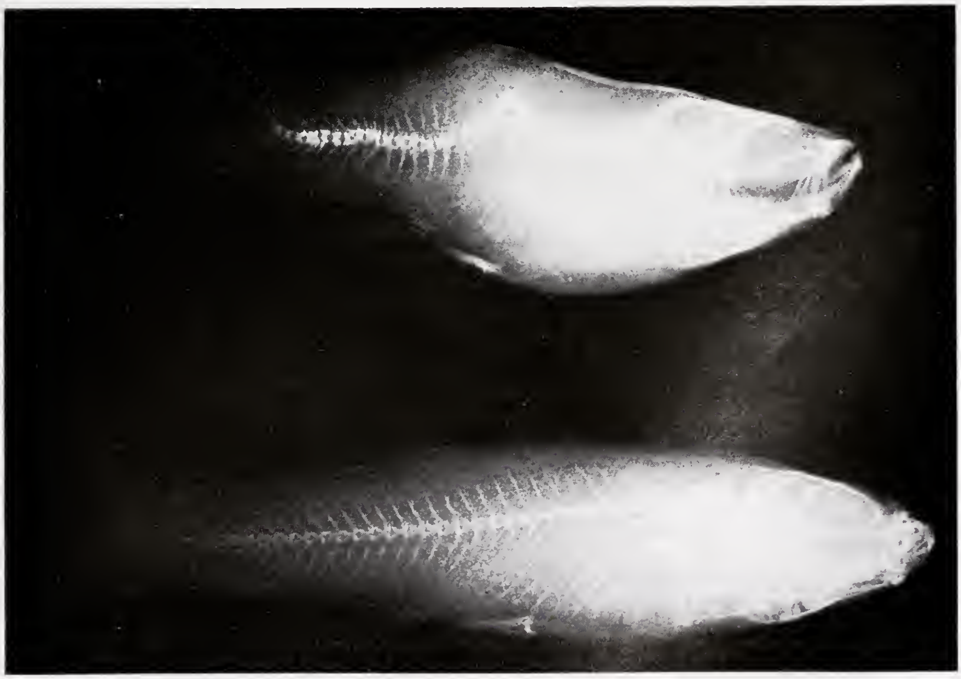


FIGURE 1. An abnormal vertebral column in *F. heteroclitus* (top) contrasted with a normal vertebral column (bottom).

between these variables; however, the concern here is not with the covariance of characters within a population, but with how this structure differs between populations.

X-ray images of skeletons were obtained using a standard medical X-ray machine and Agfa Gaevert industrial film. A focal length of one meter was used with an exposure of 20 seconds, 35 kilovolts, and 50 milliamps, and these settings produced satisfactory images for the full size range of fish measured (40–125 mm). Vertebrae were counted from X-ray images with the aid of a dissecting microscope. A fish was designated abnormal if any of the centra of its vertebral column were fractured, compound, or otherwise grossly misshapen.

RESULTS

Young specimens of *F. heteroclitus* were collected in the spring of 1970 and 1971 from both Northport and Flax Pond. In addition, samples composed of mixed year classes were collected from these localities in the fall of 1971. The number of vertebrae and incidence of vertebral abnormalities were counted from X-ray images of these population samples (Table I). The incidence of vertebral abnormalities was consistently significantly higher at Northport, and in one of the three comparisons, the mean number of vertebrae was significantly lower at Northport. Examples of vertebral abnormalities including foreshortened, asymmetrical, and fractured centra are shown in Figure 1.

Correlations of vertebral number with presence of abnormalities were calculated for three population samples from Northport. In each of the samples, there is a significant negative correlation, indicating that fish with fewer vertebrae had a higher incidence of vertebral abnormalities (Table I). Specimens of *F. heteroclitus* which develop at higher temperatures tend to have fewer vertebrae, and the incidence of vertebral abnormalities in fish with few vertebrae may indicate the limit of this developmental plasticity.

Differentiation of the Northport population with respect to number and form of vertebrae were two of many possible morphological modifications induced by environmental stress. To more accurately assess these modifications, 17 morphological characters were recorded from approximately 30 individuals from the heated locality and each of the control localities (Table II). The extent of the

TABLE I

Analysis of vertebrae in populations of F. heteroclitus. *The correlation between the incidence of vertebral abnormalities and vertebral number is represented by r.*

Collection	Sample size	Per cent with abnormal vertebrae	Mean	s.d.	s.e. of mean	r	*P*
Flax Pond 1970 Juveniles	69	4.3	33.36	0.66	0.079		
Northport 1970 Juveniles	81	15.8	33.12	0.68	0.075	-0.272	<0.05
Flax Pond 1971 Juveniles	91	2.5	33.19	0.69	0.056		
Northport 1971 Juveniles	153	21.1	33.15	0.46	0.048	-0.220	<0.01
Flax Pond 1971 Mixed	30	3.3	33.27	0.53	0.097		
Northport 1971 Mixed	30	23.3	33.30	0.75	0.136	-0.438	<0.05

TABLE II

Means, sample sizes, and standard errors of morphological variables in F. heteroclitus. *Males and females were coded 1 and 0, respectively.*

Locality (n) Variable	Flax Pond (29) Mean s.e.	Northport (25) Mean s.e.	Centerport (29) Mean s.e.	Long Island (58) Mean s.e.	South Carolina (25) Mean s.e.
Sex	0.414 ± 0.0930	0.400 ± 0.2236	0.483 ± 0.0945	0.448 ± 0.0659	0.360 ± 0.2191
Predorsal length	0.648 ± 0.0038	0.674 ± 0.0112	0.642 ± 0.0050	0.645 ± 0.0032	0.647 ± 0.0085
Head length	0.301 ± 0.0020	0.317 ± 0.0107	0.294 ± 0.0018	0.298 ± 0.0014	0.305 ± 0.0054
Snout length	0.108 ± 0.0013	0.109 ± 0.0045	0.111 ± 0.0011	0.110 ± 0.0008	0.113 ± 0.0036
Interorbital width	0.113 ± 0.0013	0.126 ± 0.0054	0.116 ± 0.0011	0.115 ± 0.0008	0.120 ± 0.0036
Dorsal fin insertion	0.152 ± 0.0030	0.157 ± 0.0063	0.165 ± 0.0029	0.158 ± 0.0022	0.153 ± 0.0076
Peduncle length	0.279 ± 0.0037	0.273 ± 0.0063	0.272 ± 0.0033	0.275 ± 0.0026	0.272 ± 0.0098
Peduncle width	0.165 ± 0.0018	0.178 ± 0.0067	0.170 ± 0.0015	0.168 ± 0.0012	0.165 ± 0.0063
Dorsal fin rays	11.690 ± 0.1226	11.560 ± 0.2911	12.379 ± 0.0917	12.034 ± 0.0885	11.640 ± 0.2853
Caudal fin rays	17.103 ± 0.1876	18.080 ± 0.7424	18.138 ± 0.2841	17.261 ± 0.1825	17.600 ± 0.3873
Anal fin rays	10.586 ± 0.1055	10.600 ± 0.2580	10.897 ± 0.9080	10.741 ± 0.0720	10.520 ± 0.2621
Pectoral fin rays	18.276 ± 0.2098	19.280 ± 0.3770	18.103 ± 0.1812	18.190 ± 0.1379	18.520 ± 0.2281
Predorsal scales	18.586 ± 0.2878	19.120 ± 0.7557	18.379 ± 0.2544	18.483 ± 0.1904	16.600 ± 0.4651
Circumpeduncular scales	19.621 ± 0.1677	20.240 ± 0.5680	20.207 ± 0.1876	19.914 ± 0.1308	19.880 ± 0.3493
Lateral line scales	34.034 ± 0.1448	34.680 ± 0.4226	34.621 ± 0.1818	34.328 ± 0.1215	34.720 ± 0.3542
Scales above lateral line	5.966 ± 0.0345	5.840 ± 0.1673	5.931 ± 0.0479	5.948 ± 0.0293	5.240 ± 0.1950
Scales below lateral line	8.552 ± 0.1536	7.160 ± 0.3578	7.931 ± 0.1484	8.241 ± 0.1136	7.560 ± 0.3672

morphological change in response to the warmer water was analyzed by principal components analysis. The three largest eigenvalues and eigenvectors, or principal axes, of the correlation matrix are presented in Table III. The sum of the first

TABLE III

Eigenvalues and loadings on principal axes from principal components analysis of morphological variation of F. heteroclitus *in a mixed sample from Flax Pond and Centerport (control environments) and Northport (heated environment).*

Eigenvalues	Principal axes		
	I 3.175	II 2.315	III 1.982
Loadings			
Sex	0.057	−0.411	0.207
Predorsal length	0.352	0.629	−0.233
Head length	0.842	0.094	0.122
Snout length	0.628	−0.221	0.385
Interorbital width	0.880	0.061	0.021
Dorsal fin insertion	0.277	−0.552	0.077
Peduncle length	−0.145	−0.422	0.068
Peduncle width	0.861	−0.149	−0.046
Dorsal fin rays	−0.065	−0.670	0.050
Caudal fin rays	0.158	0.016	−0.341
Anal fin rays	0.120	−0.308	0.114
Pectoral fin rays	0.373	0.076	−0.458
Predorsal scales	−0.228	0.097	−0.613
Circumpenduncular scales	0.124	−0.389	−0.674
Lateral line scales	0.129	−0.188	−0.678
Scales above lateral line	0.001	−0.613	−0.271
Scales below lateral line	−0.255	−0.308	0.069

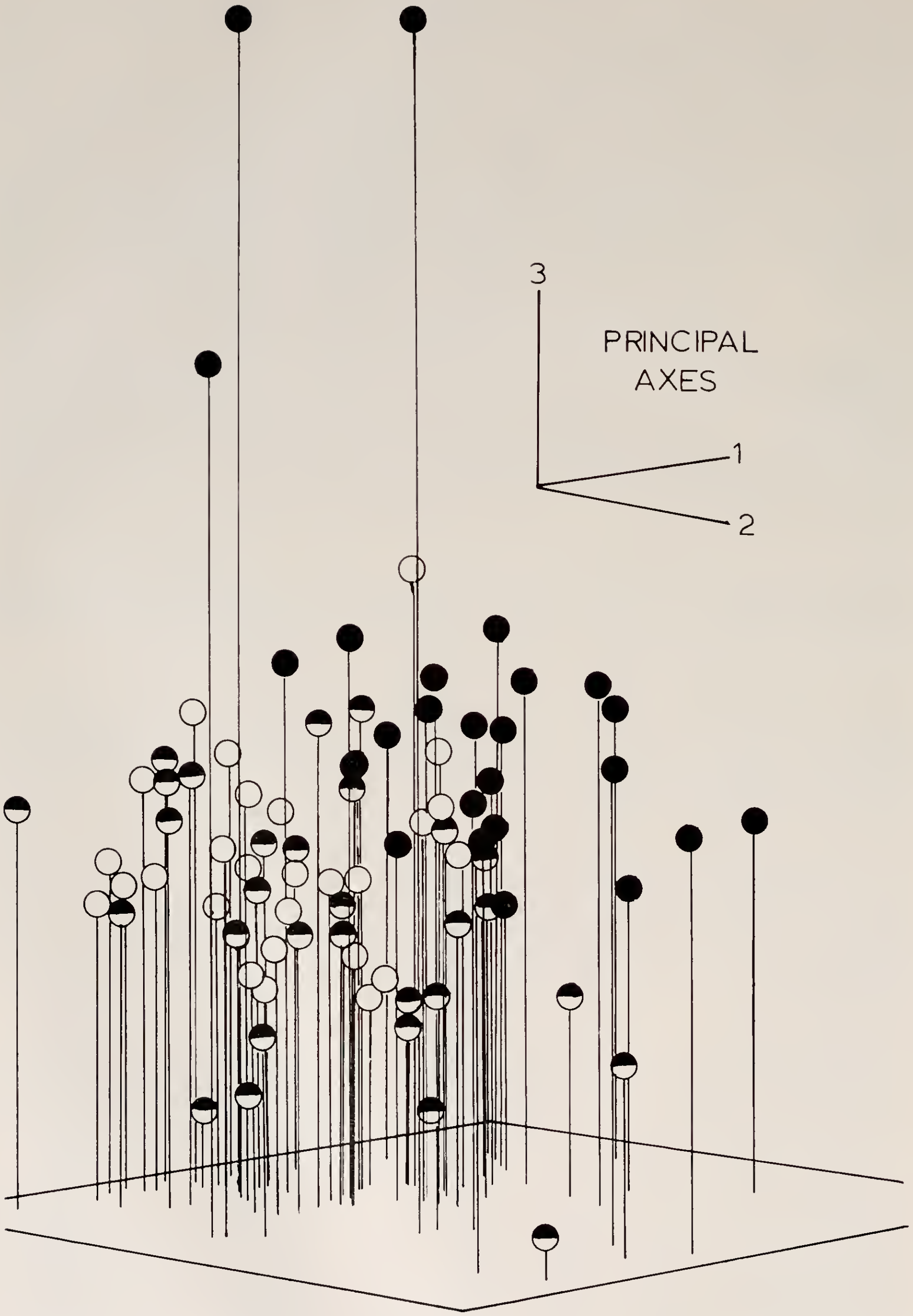


FIGURE 2. Three-dimensional representation of a principal components analysis of 78 individuals based on 17 morphological characters of *F. heteroclitus* collected from Flax Pond (half-closed circles), Northport (closed circles), and Centerport (open circles) on Long Island, New York in 1971.

three eigenvalues is 8.472, accounting for 49% of the variation in these data. Principal components analyses typically account for a higher proportion of the variance of morphometric variables with three principal axes. The low degree of explanation here seems to be due to the meristic variables, which are essentially independent of one another.

There is a considerable degree of resolution between fish from the warm and control environments. This resolution, seen in a three dimensional space defined by the first three principal axes (Fig. 2) indicates that the Northport population can be distinguished from those of surrounding control environments. Fish from the two control environments are indistinguishable in this analysis.

The characters contributing most heavily to the resolution were identified by discriminant function analysis (Table IV). Head length, snout length, interorbital width, and length of dorsal fin insertion are most important in the differentiation of the warm water population from surrounding natural populations. A frequency distribution of projections of individuals on the discriminant function (Fig. 3) shows Northport individuals are different from individuals collected from either Flax Pond or Centerport.

Morphological differentiation observed at Northport may be a product of different development rates and schedules imposed by the environment and, therefore, only a phenotypic response; or it may be a product of selection for a different phenotype in that environment and actually reflect the evolution of this population. *F. heteroclitus* is known to exhibit considerable morphological variation over its range (Brown, 1957; Relyea, 1967), and has, at times, been broken into races or subspecies, depending upon the investigator. To describe latitudinal variation in this species, a second discriminant function was constructed with both *F. heteroclitus* from naturally cold environments and *F. heteroclitus* from naturally warm environments. Fish from Centerport and Flax Pond were pooled to repre-

Table IV

Discriminant functions differentiating the morphology of F. heteroclitus *from several environments.*

Characters	Discriminant function coefficients: Heated and control environments Long Island Sound	Discriminant function coefficients: Northern and southern environments
Sex	2.619	−0.127
Predorsal length	−13.679	−9.168
Head length	−111.470	−12.287
Snout length	−5.682	37.244
Interorbital width	−18.274	−26.096
Dorsal fin insertion	31.927	−9.099
Peduncle length	−8.534	−4.683
Peduncle width	53.051	−2.183
Dorsal fin rays	−0.548	0.062
Caudal fin rays	0.480	−0.105
Anal fin rays	−0.378	0.058
Pectoral fin rays	−0.297	−0.208
Predorsal scales	1.304	−0.091
Circumpeduncular scales	−1.335	−0.161
Lateral line scales	−0.428	0.067
Scales above lateral line	9.064	0.224
Scales below lateral line	0.748	0.343

sent fish from a natural cold environment, while fish collected from Ladies Island, South Carolina (Table II) represented *F. heteroclitus* from a natural warm environment. Characters that differ between warm and cold natural environments are those associated with head shape, as well as scales above the lateral line (Table IV). Although there is some overlap of the northern and southern populations, the means of these samples are significantly different ($F_{17,65} = 8.98$, $P < 0.001$). A frequency distribution of projections of individuals on the discriminant function (Fig. 4) shows that individuals from Northport span the entire range of variation seen in the two natural environments. Again, although there is considerable variation in the morphology of the specimens from Northport, the mean for the Northport population is significantly different from both the control populations in Long Island Sound ($F_{17,65} = 3.05$, $P < 0.001$) and the population from Ladies Island, S. C. ($F_{17,32} = 3.52$, $P < 0.005$). Thus, the population exposed to the industrial thermal effluent is differentiated from surrounding control localities, and shows convergence to phenotypes abundant in naturally warm environments.

Finally, one may wonder about the relative amounts of morphological variation in the natural and stressed environments. The extreme temperatures at Northport are severe enough to produce an increased level of vertebral abnormalities, and these temperatures may lie outside the range in which the genotypes are buffered or exhibit developmental homeostasis, resulting in a greater range of morphological variation. On the other hand, the selection at Northport may have been directional to such an extent that the genetic variation underlying morpho-

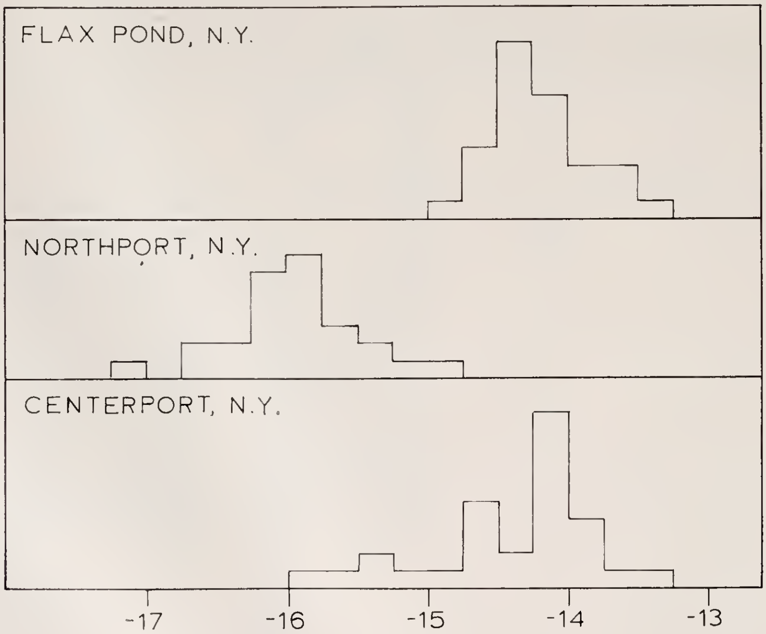


FIGURE 3. Frequency distributions of projections of individuals of *F. heteroclitus* from Flax Pond, Northport, and Centerport upon a discriminant function constructed from variance-covariance matrices of 17 morphological characters of fish from Flax Pond and Northport.

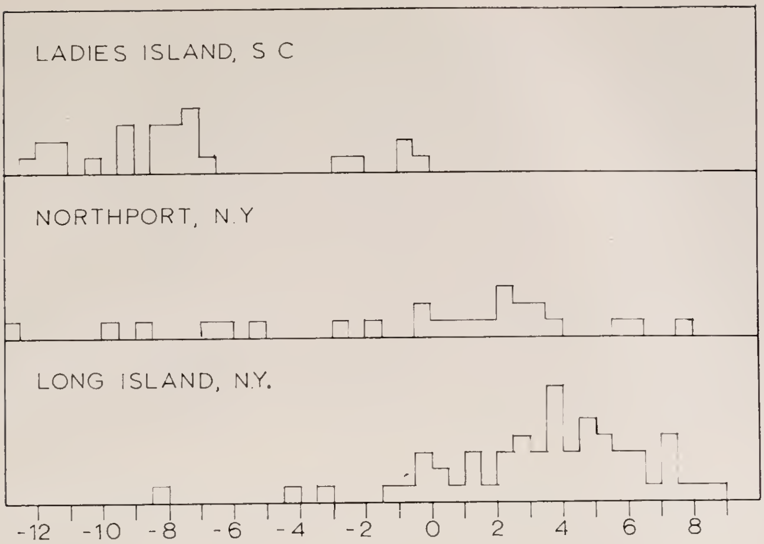


FIGURE 4. Frequency distribution of projections of individual of *F. heteroclitus* from Long Island Sound, New York, Northport, New York, and Ladies Island, South Carolina upon a discriminant function constructed from variance-covariance matrices of 17 morphological characters of fish from Long Island, New York and Ladies Island, South Carolina.

logical variation may have been depleted, leaving a restricted range of morphology. Other hypotheses may also be constructed. Resolution of these questions would ideally lie in comparisons of multivariate variances of populations, so that many characters and the correlations among them may be considered simultaneously. Unfortunately, an ideal measure of multivariate variance is not available. Sokal (1965) has suggested the generalized variance, which is the determinant of the variance-covariance matrix and is proportional to the volume of points in an n-dimensional hyperspace. Soulé (1972), in a search for a population variation parameter, finds fault with the generalized variance and offers instead a mean coefficient of variation. The mean coefficient of variation avoids problems with singular matrices, but ignores correlations between characters. Both measures are employed in this study (Table V).

In order to avoid inflated variances due only to allometric differences in samples with heterogeneous age distributions (Gould, 1966), the morphological variables have been restricted to meristic variables, determined early in development and constant thereafter. The counts employed are scale counts below the lateral line, along the lateral line, around the caudal peduncle, and fin ray counts of the dorsal, caudal, anal, and pectoral fins. Where sample sizes permit, variances are given separately for the sexes but sexual dimorphism for these seven characters is not evident. For both measures of multivariate variance, and in all comparisons, the variance is greater at Northport than in any of the control localities (Table V). The difference is most pronounced when measured by the generalized variance, which utilizes both the variances of the characters and the covariances among them.

TABLE V

Multivariate variances for seven meristic characters of F. heteroclitus *from control and heated (Northport) localities.*

Sample	Sample size	Generalized variance	Mean coefficient of variance
Samples sorted by sex			
Flax Pond males	88	0.080	5.77
Flax Pond females	178	0.056	5.58
Northport males	111	0.202	6.32
Northport females	195	0.136	6.04
Samples including both sexes			
Flax Pond	29	0.024	5.56
Centerport	30	0.023	5.79
Ladies Island, S. C.	25	0.003	5.12
Northport	24	0.052	6.36

DISCUSSION

Gabriel (1944) performed experiments upon subsets of sibships to determine which, if any, factors influence the number of vertebrae in *F. heteroclitus*. The number of vertebrae were influenced by genetic factors, temperature dependent factors, and temperature independent factors. Sibs raised at different temperatures had different numbers of vertebrae, with vertebral number decreasing with increasing temperature of development. Although this result is straightforward, mechanisms underlying temperature dependent shifts in meristic characters of teleosts are complex (Fowler, 1970). Gabriel also found a phenomenon, presumably with a genetic basis, that interfered with predictions of the response of vertebral number to temperature. Some sibs responded to temperature in the manner described above, but in others the response was mitigated. Tåning (1952) found V-shaped rather than linear functions of the mean count of a meristic variable with respect to temperature during development, and it is not implausible that the underlying mechanism may apply to characters with continuous variation as well. Barlow (1961) proposed that temperature dependent shifts in meristic characters are the product of two separate temperature dependent rates, the rate of growth or increase in mass, and the rate of development or differentiation. If one rate is more temperature dependent than the other, different counts will result at different temperatures of development. A V or inverted V-shaped function will result if one of the rates has an inflection point.

Thus, the determination of the state of a meristic variable is complex, and reliable quantitative predictions from knowledge of temperature might be too much to expect. Although Gabriel (1944) found vertebral number to be a linear function of temperature of development, the possibility that other meristic counts and variables with continuous variation might have more complicated response functions to temperature, or no response at all, cannot be ruled out. Therefore, knowledge of processes underlying the determination of states of meristic or continuous characters is not sufficient to allow us to predict the magnitude of a morphological change for a given environmental perturbation, or perhaps even its direction. From Gabriel's work (1944), however, one would predict that vertebral number would be lower in the elevated temperatures at Northport, if they were different at all. In one of three comparisons (Table I), significantly fewer vertebrae were found in fish from Northport.

A prediction may also be based upon the latitudinal variation in morphology, which is presumably a response to temperature. If the morphological differentiation at Northport is predominantly a response to temperature, the changes occurring there should make that population resemble the morphology of southern populations. Support for this hypothesis is seen in Figure 4.

Evidence that the environment at Northport imposes a stress upon *F. heteroclitus* is provided by the elevated frequency of vertebral abnormalities observed there. A similar observation was made by Hubbs (1959), who found vertebral abnormalities to be common in the mosquito fish, *Gambusia affinis,* living in natural hot springs in Texas and Mexico. Higher incidence of vertebral abnormalities in fish with fewer vertebrae (Table I) suggests that fish marked with a vertebral abnormality are individuals whose tolerance limits had been exceeded, and whose canalization has been disrupted. The fish with severe abnormalities, as well as the individuals that disappeared before the time of sampling, may be carriers of different genes, or different combinations of genes, than those that seemed to have developed normally. If many genes contribute to set the physiological environment of the fish and those genes have pleiotropic effects, the opportunity for genetic and genetically based morphological divergence of this population is great. The incidence of severe vertebral abnormalities marks a clear opportunity for selection to act, and an analysis of enzyme polymorphisms (Mitton, 1973; Mitton and Koehn, 1975) provides evidence that selection has operated. A large gravid female may carry several hundred eggs at one time and may have two spawning periods, each several months in length (Mathews 1938). This high fecundity, and high degree of genetic variability discovered (Mitton 1973; Mitton and Koehn 1975) may permit rapid differentiation in a severe environment.

Specimens of *F. heteroclitus* at Northport generally have longer and wider heads than those of *F. heteroclitus* at surrounding control localities, and at the same time, have more and larger gill filaments (G. Williams, State University of New York at Stony Brook, personal communication), providing them with greater gill surface area. The need for greater gill surface at this locality is clear. Metabolic rates of these fishes are enhanced by higher temperatures, but the amount of oxygen dissolved in the water is likely to be lower. Thus, the larger head dimensions in warm water populations are a reflection of greater gill surface area, which is demanded by the greater need of oxygen combined with its lower availability.

Breeding studies in fishes, including *F. heteroclitus*, have amply demonstrated that morphology has a genetic basis (Gabriel, 1944; Hagen, 1973) and is influenced by the environment. Complex mechanisms underlying morphology (Gabriel, 1944; Tåning, 1952; Barlow, 1961; Fowler, 1970), however, leave little hope of predicting morphological variation in given circumstances or understanding of whether changes observed are strictly developmental phenomena and/or have a substantial genetic component. Discriminant function analysis has shown the morphology of *F. heteroclitus* at Northport to be different than morphology in surrounding localities and has revealed a possible convergence towards the phenotype of southern populations, but there are no direct data that show this phenotypic convergence to have a genetic basis.

A multivariate analysis of both morphological and protein phenotypes performed upon these population samples (Mitton, in preparation) reveals that morphological variation is not independent of protein variation. In addition, analyses of protein variation identified genetic divergence of the artificially-heated population from surrounding control localities (Mitton, 1973; Mitton and Koehn, 1975). If allelic frequencies at polymorphic loci controlling morphological characters have been altered to the same extent as the allelic frequencies of loci coding for enzymatic proteins, much of the morphological differentiation observed here could have a genetic basis.

Several mechanisms may be presented to explain the greater range of morphological variation at Northport than in the control localities; and although none can be firmly rejected, some are more plausible than others. Soulé (1971) and Morris and Kerr (1974) have reported correlations between genic heterozygosity as measured by protein polymorphisms and some measure of morphological variation. Levels of heterozygosity tend to be higher at Northport than at Flax Pond (Mitton and Koehn, 1975), but the difference is slight and is probably not sufficient to explain the two-fold difference in variation detected by the generalized variance (Table V). Fisher (1930) demonstrated that the rate of evolution of a population was proportional to its variance in fitness, and the Northport population is presented as a population adapting to a new environment, but it has not been demonstrated that variation in meristic characters of *Fundulus* is adaptive variation (Tables III, IV, V). Meristic variation has been implicated in selection (Fox, 1975 and references therein), but the data presented here neither suggest nor deny an adaptive function of this variation. Although rigorous selection is generally presented as a force decreasing variation, Warburton (1967) has shown that if rare phenotypes are favored, selection will actually result in an increase in variation. The morphology of northern control localities is different from the morphology typical at the southern control locality (Fig. 4), and the morphology of *F. heteroclitus* at Northport seems to be converging toward the southern morphology, so the situation envisioned by Warburton (1967) seems to be realized at Northport. Although disruption of developmental homeostasis (Table I) may contribute to the variation at Northport, the large amount of morphological variation may also be an adaptive concomitant of selection for an unsual phenotype.

This research was supported by National Science Foundation grant GB–25343 and PHS Career Award GM–28963 to R. K. Koehn, by a grant in aid from the Theodore Roosevelt Memorial Fund of the American Museum of Natural History to J. B. Mitton, and by NIH training grant GM 400360–24257 3253 to the Department of Genetics, University of California, Davis.

Summary

Populations of *Fundulus heteroclitus* (Cyprinodontidae), a coastal marine fish, were studied in control and artificially heated environments on the north shore of Long Island to determine patterns of variation in morphology and the extent to which this variation reflected adaptation to environmental characteristics.

Principal components and discriminant function analyses were used to analyze variation in and among seventeen morphological characters. Fishes living in water artificially heated by a power plant exhibited marked divergence from control populations in head morphology, and convergence with a population sampled at more southern latitudes. Hence, these differences were interpreted as adaptations to warm environments. Greater morphological variation is detected at the heated locality than at control localities, and this may be partially due to a breakdown in developmental homeostasis, and partially due to selection favoring phenotypes that are rare in this environment.

LITERATURE CITED

Atchley, W. R., C. T. Gaskins, and D. Anderson, 1976. Statistical properties of ratios. I. Empirical results. *Syst. Zool.,* **25**: 137–148.

Barlow, G. W., 1961. Causes and significance of morphological variation in fishes. *Syst. Zool.,* **10**: 105–117.

Brown, J. L., 1957. A key to the species and subspecies of the cyprinodont genus *Fundulus* in the United States and Canada, east of the Continental Divide. *J. Wash. Acad. Sci.,* **47**: 69–77.

Fisher, R. A., 1930. *The genetical theory of natural selection.* Clarendon Press, Oxford, 272 pp.

Fowler, J. A., 1970. Control of vertebral number in teleosts—an embryological problem. *Quart. Rev. Biol.,* **45**: 148–167.

Fox, S. F., 1975. Natural selection on morphological phenotypes of the lizard *Uta stansburiana. Evolution,* **29**: 95–107.

Gabriel, M. L., 1944. Factors affecting the number and form of vertebrae in *Fundulus heteroclitus. J. Exp. Zool.,* **95**: 105–143.

Gould, S. J., 1966. Allometry and size in ontogeny and phylogeny. *Biol. Rev.,* **41**: 587–640.

Gould, S. J., 1971. Geometric similarity in allometric growth: a contribution to the problem of scaling in the evolution of size. *Amer. Natur.,* **105**: 113–136.

Hagen, D. W., 1973. Inheritance of numbers of lateral plates and gill rakers in *Gasterosteus aculetus. Heredity,* **30**: 303–312.

Hubbs, C., 1959. High incidence of vertebral deformities in two natural populations of fishes inhabiting warm springs. *Ecology,* **40**: 154–155.

Hubbs, C. L. and K. F. Lagler, 1949. Fishes of the Great Lakes region. *Cran. Inst. Sci. Bull.,* **26**: 1–186.

Matthews, S. A., 1938. A seasonal cycle in the gonads of *Fundulus. Biol. Bull.,* **75**: 66–74.

Mitton, J. B., 1973. Genetic and morphological adaptation to thermal stress in the coastal marine fish *Fundulus heteroclitus. Ph.D. thesis, State University of New York at Stony Brook,* Stony Brook, New York, 115 pp. (*Diss. Abstr.,* **34**: 4846; order no. 78–08943).

Mitton, J. B., and R. K. Koehn, 1975. Genetic organization and adaptive response of allozymes to ecological variables in *Fundulus heteroclitus. Genetics,* **79**: 97–111.

Morris, L. N. and B. A. Kerr, 1974. Genetic variability in Macaques: correlation between biochemical and dermatoglyphic systems. *J. Hum. Evol.,* **3**: 223–235.

Relyea, K. G., 1967. Systematic study of two species complexes of brackish water *Fundulus* (Pisces: Cyprinodontidae). *Ph.D. thesis, Tulane University,* New Orleans, Louisiana, 214 pp. (*Diss. Abstr.,* **28**: 3109; order no. 67–17942).

Sokal, R. R., 1965. Statistical methods in systematics. *Biol. Rev.,* **40**: 337–391.

Soulé, M., 1971. The variation problem: the gene flow-variation hypothesis. *Taxon,* **20**: 37–50.

Soulé, M., 1972. Phenetics of natural populations. III. Variation in insular populations of a lizard. *Amer. Natur.,* **106**: 429–446.

Tåning, A. V., 1952. Experimental study of meristic characters in fishes. *Biol. Rev.,* **27**: 169–193.

Warburton, F. E., 1967. Increase in the variance of fitness due to selection. *Evolution,* **21**: 197–198.

Reference: *Biol. Bull.* **151**: 560–573. (December, 1976)

DEVELOPMENT AND METAMORPHOSIS OF THE SEA-STAR, *ASTROPECTEN SCOPARIUS* VALENCIENNES

CHITARU OGURO, MIÉKO KOMATSU AND YASUO T. KANO

Department of Biology, Toyama University, Toyama 930, Japan and Uozu Aquarium, Uozu, Toyama 937, Japan

The development of sea-stars has been reported in a number of species (reviewed by MacBride, 1914; Hyman, 1955; Dan, 1957; Hayashi, 1972). However, it seems that enough information has not been available on the entire process of the development and the metamorphosis to make it possible to discuss the phylogenetic significance of developmental features, as well as of the post-metamorphic growth.

The development of sea-stars is generally divided into two types, the indirect and the direct. In the former, the embryo develops into brachiolaria after passing through bipinnaria as reported in *Asterias rubens, Asterias amurensis* and *Acanthaster planci* (Gemmill, 1914; Dan, 1957; Henderson and Lucas, 1971). In the direct development, only brachiolaria appears, and the bipinnaria stage is entirely lacking. This type of development has been reported in a number of species as exemplified by *Asterina gibbosa, Henricia sanguinolenta, Certonardoa semiregularis* and *Echinaster echinophorus* (MacBride, 1896; Masterman, 1902; Hayashi and Komatsu, 1971; Atwood, 1973). However, development of the species belonging to the genus *Astropecten* may not be exactly classified into either of the two types mentioned above, since they undergo metamorphosis while larvae are pelagic, usually as a bipinnaria, and lack the brachiolaria stage completely. The development of *Astropecten aranciacus* may illustrate a typical process of abrachiolarian type of development (Hörstadius, 1939). Mortensen (1921, 1937) gave brief notes on metamorphosing larvae of two Japanese astropectens, *Astropecten scoparius* and *Astropecten polyacanthus* and showed that the metamorphosis of these species may occur without passing through brachiolaria stage. Furthermore, brief accounts were recently presented on the early development of these two astropectens (Komatsu, 1973; Oguro, Komatsu and Kano, 1975). However, the details of the entire process of the development of these two species remain unknown.

Since there is a noticeable feature in the development of *Astropecten* species as noted above, it is important to learn the development of this group in detail, especially its metamorphosis, for a thorough understanding of the significance of development in sea-stars.

The writers have had opportunities to observe the development of *Astropecten scoparius* in the last few years, and the following is a description of the entire process of its development in terms of external morphology and skeletal system formation.

MATERIALS AND METHODS

Adults of *Astropecten scoparius* Valenciennes were collected along the coast of Toyama Bay, Sea of Japan. The present species is one of the most common

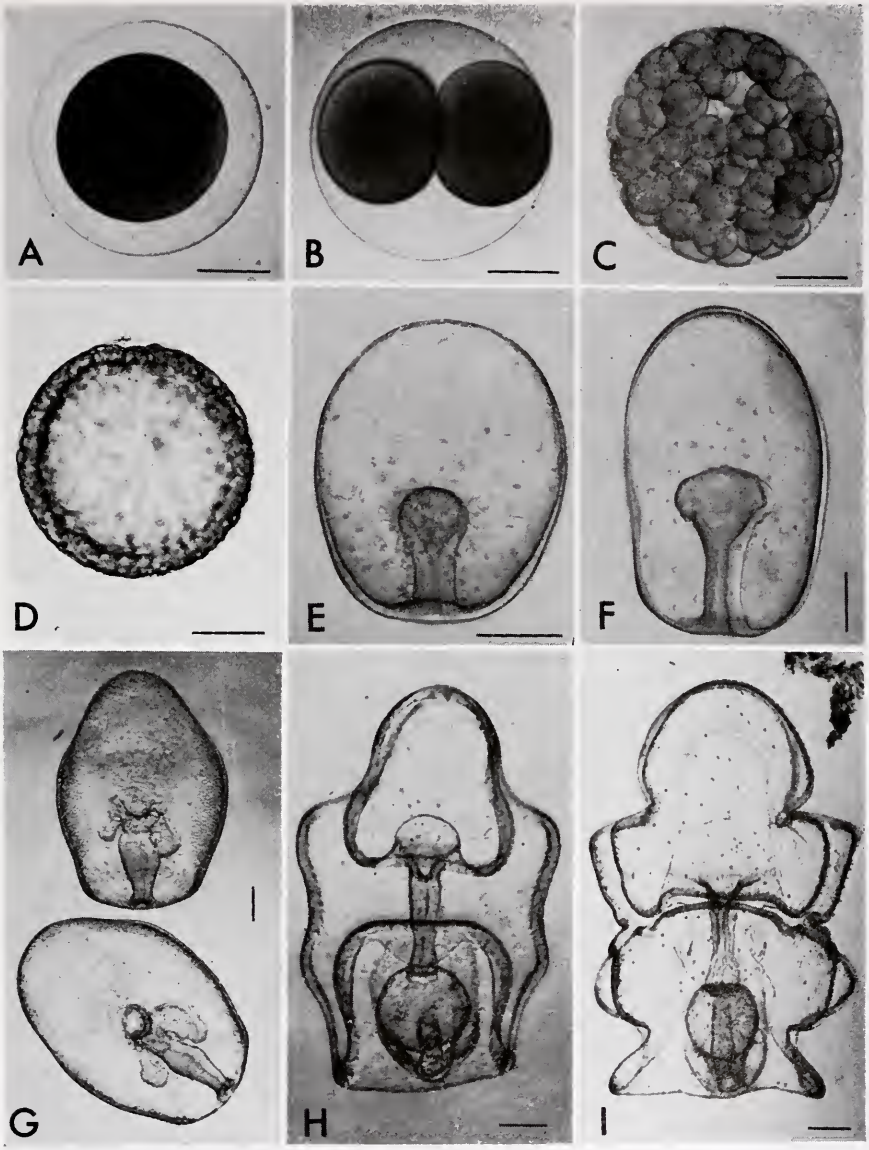


FIGURE 1. Development of *Astropecten scoparius.* All pictures show living specimens; scale = 100 μ: A) fertilized egg with complete fertilization membrane; B) two-cell stage; C) early blastula; D) coeloblastula; E) gastrula, 15 hours after insemination; F) gastrula, 20 hours after insemination; G) early bipinnaria, 35 hours after insemination, ventral view; H) bipinnaria, 48 hours after insemination, ventral view; and I) bipinnaria, 80 hours after insemination, ventral view.

sea-stars in Japan. This species is gonochristic. The gonad, in both sexes, is composed of tufted tubules and restricted to the base of each arm. Each gonad is furnished with a gonoduct which opens on the aboral side of the arm base. The breeding season in Toyama Bay is estimated to be July–August.

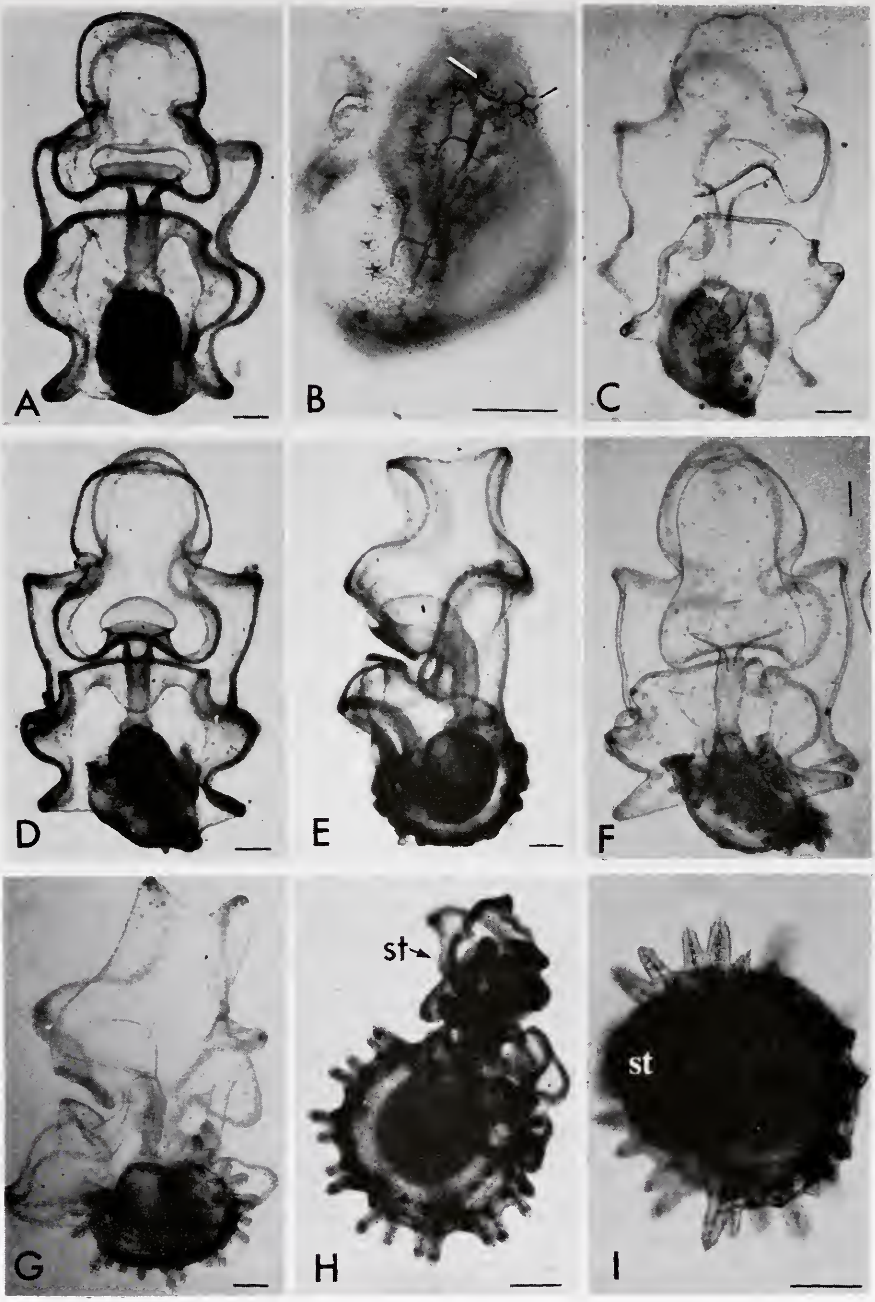


FIGURE 2. Development of *Astropecten scoparius.* All pictures show living specimens; scale = 100 μ: A) bipinnaria, 7 days after insemination and at the onset of metamorphosis, ventral view; B) enlarged picture of the posterior portion of the metamorphosing bipinnaria, the same specimen as shown in Figure 4B—short and long arrows point to rudimental

Observations were mostly made on materials obtained by artificial fertilization as will be described below. Ovaries were dissected out and placed in petri-dishes, in which a seawater solution of 1-methyladenine was applied to induce the completion of maturation and spawning (see Kanatani, 1969). Twenty to 60 minutes thereafter, mature ova were released from the ovaries. The spawned ova were removed and washed thoroughly. A few drops of sperm suspension were applied to the dishes containing the mature ova. In addition to the artificially raised embryos, observations on the later development were supplemented by materials from natural spawning in aquaria of Uozu Aquarium, and from collection in the field. Standard temperature for the development in the laboratory was 25° C. For the observations of external morphology, specimens were studied by light microscope or phase-contrast microscope. Examinations of the skeletal system were mostly performed after treatment with KOH solution. In addition, skeletal plates of the juveniles were observed by scanning electron microscope after the treatment by KOH and sonication. Sea water used in the standard observations in the laboratory was obtained from the coast of Toyama Bay and filtered before use. Average salinity was 34‰ and pH was 8.3.

Observations and Results

The mature ova are spherical, translucent, approximately 230 μ in diameter, and are enclosed in a jelly layer about 1 μ thick. They are heavier than sea water. About 5 minutes after insemination, the fertilization membrane began to elevate, and 40 minutes thereafter the process was completed with a perivitelline space 50 μ in height (Fig. 1A). One hour after insemination, the first cleavage occurred through the animal-vegetal axis (Fig. 1B). The embryos were in the 4-cell stage 100 minutes after insemination, and in the 32-cell stage 150 minutes after insemination. The cleavage is of the holoblastic radial type (Fig. 1C), and they developed into coeloblastulae 5 hours after insemination (Fig. 1D). Then, the wrinkled blastula stage began and it continued for about 4 hours. The details of the wrinkled blastula stage of the present species have been described previously (Komatsu, 1973). Nine and a half hours after insemination, gastrulation by invagination took place from the vegetal pole. Ten hours after insemination, early gastrulae began to rotate within the fertilization membrane, and they hatched 40 minutes thereafter. Gastrulae just after hatching were 250 μ in length. They gradually elongated along the archenteric axis, and 15 hours after insemination they measured 350 μ in length and 270 μ in width (Fig. 1E). The blind end of the archenteron expanded, showing the differentiation of the future coelomic pouches. Mesenchymal cells were set free into the blastocoel. Five to

---

madreporic plate and hydropore, respectively; C) metamorphosing bipinnaria, slightly later than that shown in Figure 4B, ventro-lateral (right) view; D) metamorphosing bipinnaria, 10 days after insemination, the same stage as shown in Figure 4D, E and F, (ventral view); E) lateral (left) view of the same specimen shown in Figure 2D; F) metamorphosing bipinnaria, 12–13 days after insemination, ventral view; G) lateral view (left) of the same specimen shown in Figure 2F; H) metamorphosing bipinnaria with shrunken stalk (st), future aboral side view; and I) metamorphosing juvenile with rudimental larval stalk (st), future oral side view.

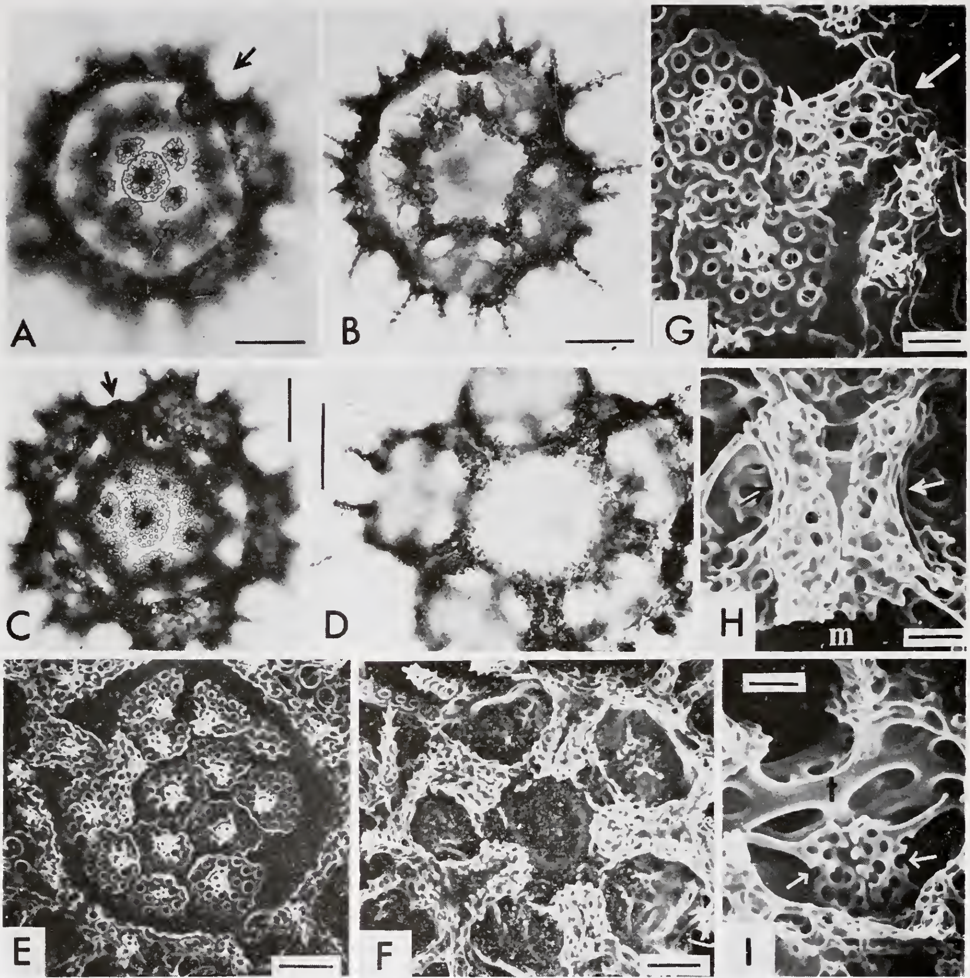


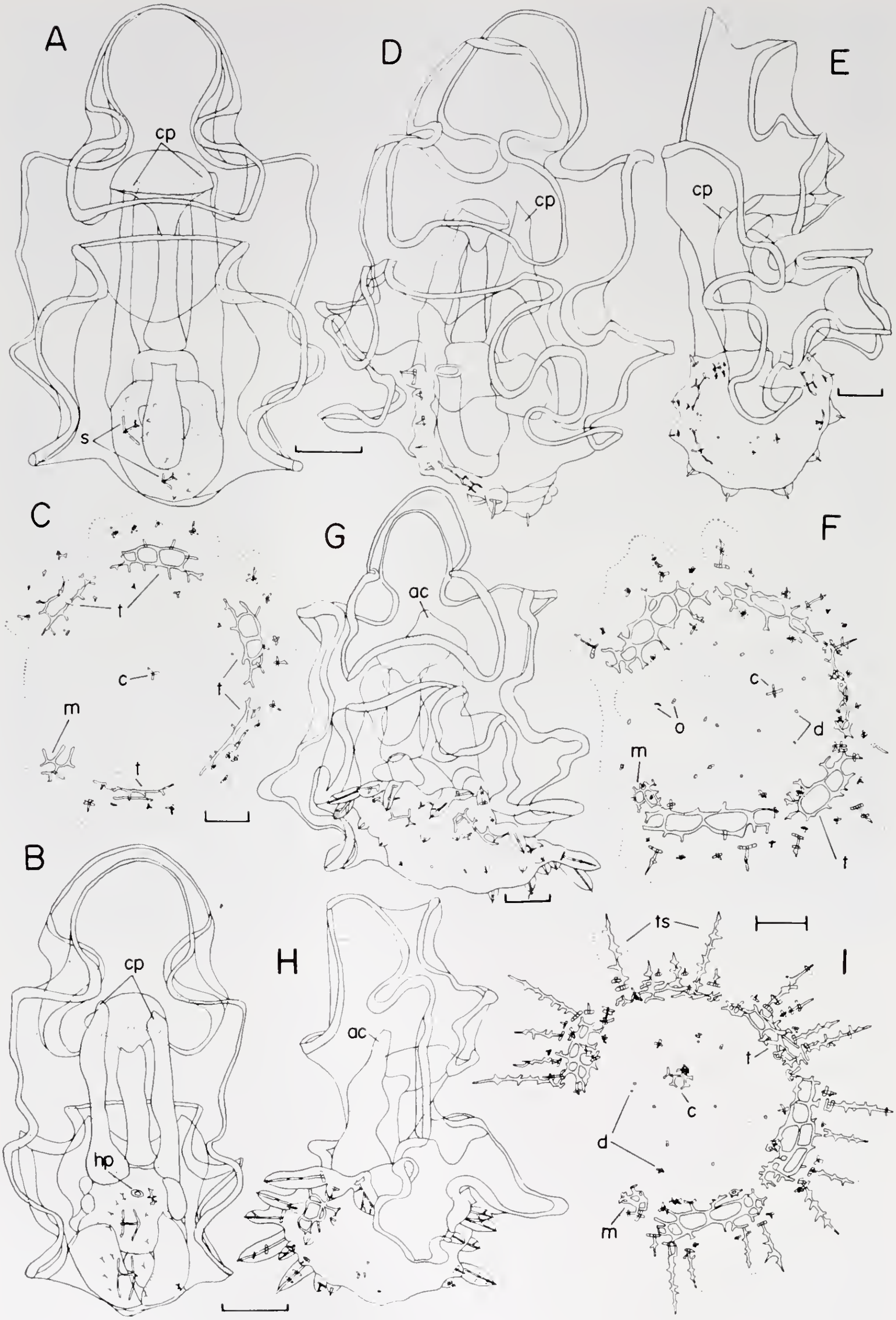
FIGURE 3. Development of *Astropecten scoparius*. All specimens were preserved and treated with KOH solution. Scale = 100 μ in A–D: A) aboral skeletal plates of a juvenile, 18 days after insemination and immediately after metamorphosis—arrow (upper right) shows madreporic plate; B) oral skeletal plates of the same specimen shown in Figure 3A; C) aboral skeletal plates of a juvenile, 23 days after insemination—arrow shows madreporic plate; and D) oral skeletal plates of the same specimen shown in Figure 3C. Figure 3 E–I, shows scanning electron microscopy. Scale = 50 μ in E and F; 25 μ in G–I: E) aboral skeletal plates of a juvenile, one month after insemination; F) oral skeletal plates of the same specimen shown in Figure 3E; G) madreporic plate (arrow) of a juvenile, 1 month after insemination; H) a pair of oral plates (arrows) of a juvenile, one month after insemination (m shows mouth); and I) a pair of ambulacral plates (arrows) of a juvenile, one month after insemination (t, terminal plate).

7 hours thereafter, a pair of the rudimental coelomic pouches was distinguishable at both sides of the top of the archenteron (Fig. 1F). The length and the width of this specimen were 600 μ and 450 μ, respectively. At this time, the oral depression was recognized on the future ventral side of the larva. Thirty-five hours

after insemination, 2 ciliary bands became obvious and the larva could be called a bipinnaria. The archenteron began to differentiate into intestine, stomach and esophagus which opens at the oral depression. The hydropore opened in the left coelomic pouch, which is at this time larger than the right one (Fig. 1G). Forty-eight hours after insemination, the length and the width of the bipinnaria were 850 μ and 600 μ, respectively. The left and the right coelomic pouches were separated from the digestive tract, and each coelomic pouch was constricted at the middle portion into two parts, the anterior (hydrocoel) and the posterior (stomatocoel) (Fig. 1H). Eighty hours after insemination, the bipinnaria grew to a considerable size, 1,000 μ in length and 650 μ in width, and the ventral horn was well-developed between the intestine and the stomach (Fig. 1I).

About one week after insemination, the posterior portions of the bipinnariae, which were reared in the aquaria, became swollen and the gastric portion became rather opaque (Fig. 2A). Shortly thereafter, several spicules were formed on the posterior portion of the bipinnariae (Fig. 4A). These spicules were not larval ones, but they were the rudiments of the future terminal plates of the juveniles. Average length of the bipinnariae of this stage was 1,150 μ, although remarkable variation exists in the progress of the development and in the size of the larvae in the present species. The larvae of the present material were far larger than those reported by Mortensen (1937) as a fully grown bipinnaria (600 μ in length) of this species. Primordia of 5 hydrolobes then appeared in the posterior end of the left hydrocoel. Metamorphosing bipinnaria shown in Figure 4B was one day after that shown in Figure 4A. Enlarged pictures of the spicules of this specimen are shown in Figure 2B. Thereafter, the posterior part of the larvae was transformed progressively into a subpentagonal sea-star shape, while the anterior part was kept almost unchanged for a few days (Fig. 2C). The aboral skeletal system at this stage was composed of one central plate and one madreporic plate, in addition to five terminal plates, each furnished with several spines (Fig. 4C). About ten days after insemination, the disk of the future juvenile sea-star was well marked from the bipinnaria stalk and the aboral portion of the future disk could be observed from the right side of the larva (Figs. 2D, 2E, 4D, 4E). In this stage, the rudiments of five pairs of oral plates and those of several dorsal plates around the central plate were formed on the future oral and aboral side, respectively (Fig. 4F). Two days thereafter, the hydrocoelomic pouches in both sides extended so as to contact each other at the anterior ends (Figs. 4G, 4H). At this time, two spines on the edge of the terminal plates became extremely long (Fig. 4I). Five pairs of the rudimental ambulacral plates were set on the future oral side of the juveniles (Fig. 5A). About 12–13 days after insemination, the anterior part of the larva still kept the typical form of the bipinnaria of this species (Figs. 2F, 2G).

About two weeks after insemination, the stalk, the anterior portion of the bipinnaria, began to shrink rapidly, and thus the progress of the metamorphosis seemed to be accelerated at this time (Fig. 2H). On the other hand, Mortensen (1921) reported that the metamorphosis of the present species is initiated three weeks after insemination. The metamorphosing bipinnariae with shrunken stalk sank to the bottom; then the ciliary bands became inconspicuous due to the extreme reduction of the stalk (Figs. 5B, 5C). The reduced stalk disappeared

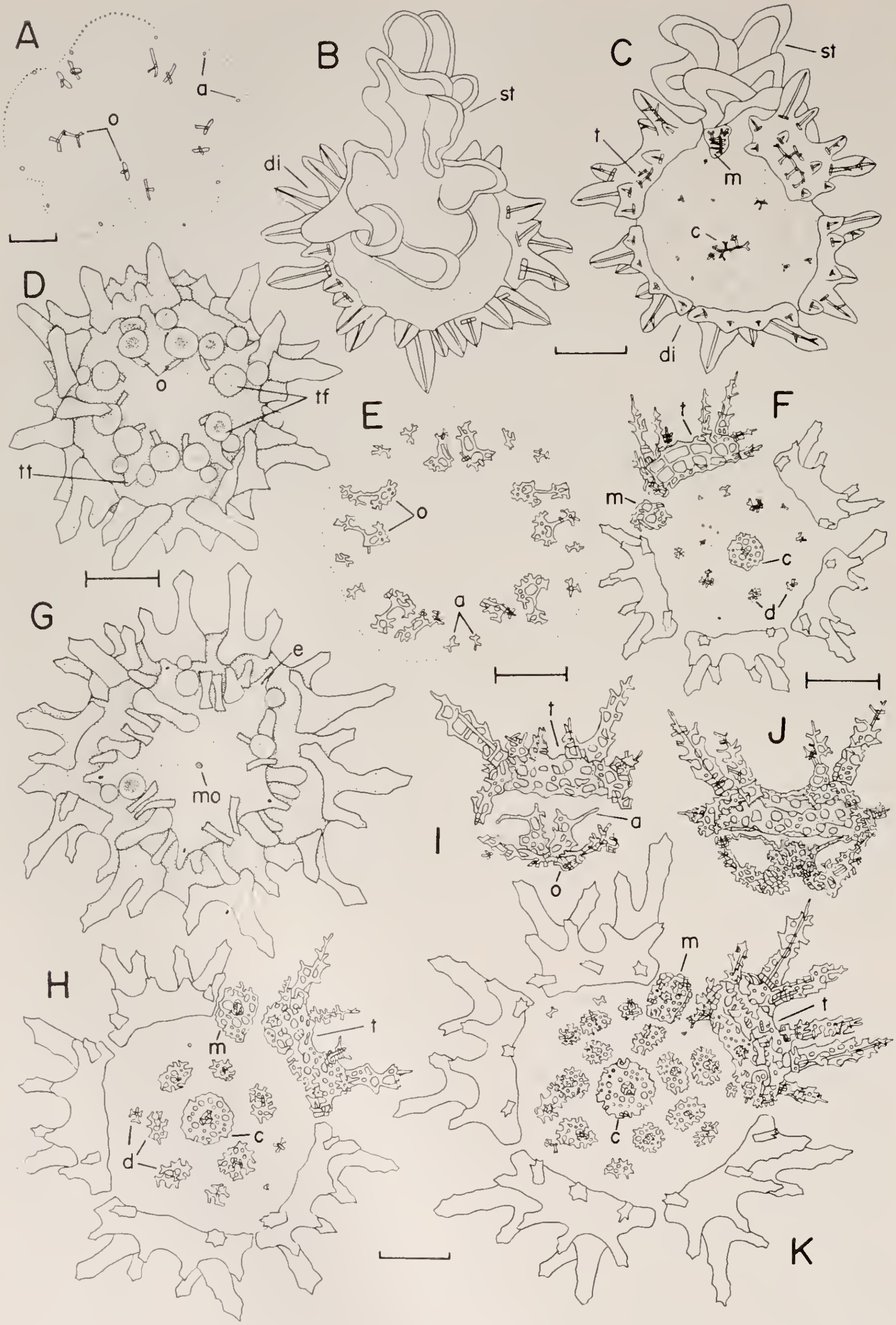

A
cp
s
D
cp
E
cp
C
t
t
c
m
t
G
ac
F
c
o
d
m
t
B
cp
hp
H
ac
ts
I
t
c
d
m

within a few days (Fig. 2I). Then the primordia of two pairs of tube-feet and of a single terminal tentacle appeared on each hydrolobe. About 16 days after insemination, the metamorphosing juveniles could move slowly on the substratum by means of their tube-feet (Fig. 5D). It is worth notice that the tube-feet of these juveniles are suckered, while the tube-feet of the adult lack suckers. Examination of the serial sections of these juveniles showed that they lack anus as in the adults. The diameter of these juveniles was about 500 μ including marginal spines. The skeletal system of this stage is shown in Figures 5E and 5F.

About 18 days after insemination, the mouth opened and thus metamorphosis was completed (Fig. 5G). Juveniles immediately after metamorphosis were white in color with a pale brown portion at the center of the disk and about 600 μ in diameter. Each arm bore two pairs of the tube-feet and a terminal tentacle with an eye-spot of red color. On the aboral side of the disk, about 10 plates were recognized in addition to one central plate and one madreporic plate (Figs. 3A, 5H.) However, radial plates were not distinguishable from the interradial plates because of their irregular arrangement. Figures 3B and 5I represent the skeletal system of the oral side of the juvenile just after metamorphosis. The features of the skeletal system of newly metamorphosed juveniles of the present species are very similar to those of *Astropecten aranciacus* and *Astropecten latespinosus* (Hörstadius, 1939; Komatsu, 1975a). About five days after the completion of metamorphosis, juveniles grew to about 700 μ in diameter. During these days, skeletal formation progresses further (Figs. 3C, D; 5J, K). Scanning electron microscopy illustrates the stereo-structure of the skeletal system of juveniles one month after the completion of metamorphosis (Fig. 3E–I).

After the completion of laboratory observations, a field survey of the juveniles was carried out by SCUBA diving in the area where the adults inhabit. The following descriptions are based on these collections. The smallest specimens collected in October and November were 2.2 and 1.7 mm in R (the distance from the center of the disk to the arm tip), respectively. Since the breeding season of the present species in Toyama Bay is presumed to be July–August as noted before, the juveniles grew from 600 μ to 3.5–4.0 mm in diameter during these two to three months.

FIGURE 4. A) Metamorphosing bipinnaria, 7 days after insemination, ventral view; cp, coelomic pouch; s, spine; scale = 150 μ. B) Metamorphosing bipinnaria, one day after that shown in Figure 4A, dorsal view: cp, coelomic pouch; hp, hydropore; scale = 150 μ. C) Skeletal system of metamorphosing bipinnaria, the same stage as shown in Figure 2C, view from the future aboral side of the juvenile: c, central plate; m, madreporic plate; t, terminal plates; scale = 50 μ. D) Metamorphosing bipinnaria, 10 days after insemination, the same stage as shown in Figure 2D and E: cp, coelomic pouch; scale = 150 μ. E) Lateral (right) view of the same specimen shown in Figure 4D: cp, coelomic pouch; scale = 100 μ. F) Skeletal system of metamorphosing bipinnaria, the same stage as shown in Figure 4D and E, view from the future aboral side of the juvenile: c, central plate; d, dorsal plates; m, madreporic plate; o, oral plates; t, terminal plate; scale = 50 μ. G) Metamorphosing bipinnaria, 12 days after insemination, ventral view; ac, anterior coelom; scale = 100 μ. H) Lateral (right) view of the specimen shown in Figure 4G: ac, anterior coelom; scale = 150 μ. I) Skeletal system of the dorsal side of metamorphosing bipinnaria, the same stage as shown in Figure 4G and H, view from the future aboral side of the juvenile: c, central plate; d, dorsal plates; m, madreporic plate; t, terminal plate; ts, spines of the terminal plates; scale = 50 μ.


A
a
o
B
st
di
C
st
t
m
c
di
D
o
tf
tt
E
o
a
F
t
m
c
d
G
e
mo
I
t
a
o
J
H
m
t
c
d
m
t
c
K

Although it has been well known that some diagnostic features are not developed in small-sized specimens of sea-stars, no substantial studies have been previously carried out. In the present study, the development of some diagnostic features was observed on the post-metamorphosed specimens. In the adults, superomarginal plates are each furnished with one long spine excepting the interbrachial arch, and this is designated as one of the diagnostic features of the present species. However, no spines are found in the superomarginal plates in specimens smaller than 9.0 mm in R or having less than 30 pairs of tube-feet in one arm. Studies were then extended to the R/r value (a ratio between R, the distance from the center of the disk to the arm tip, and r, the distance from the center of the disk to the middle of the interbranchial margin). The R/r value is used as a general mark in the description of asteroids, and 3–4 is noted in the present species. Examination of the present material showed that specimens smaller than 9 mm in R show less than 3.0 or even less than 2.0. A R/r value showing more than 3 is found in specimens larger than 9 mm in R. Observations were also made on the paxillae. Paxillae of specimens ranging from 4 to 9 mm in R are furnished with 5–6 peripheral spines but lack central spines. Central spines are formed in specimens larger than 9 mm in R. Then paxillar spines increase in number as the juveniles grow. In a specimen reaching 14.6 mm in R, the majority of the paxillae are furnished with 4 central and 12 peripheral spines. Since in fully grown adults, the largest paxillae are furnished with 10–15 central and 15–18 peripheral spines (Hayashi, 1973), formation of paxillae is completed more than two months after metamorphosis. These facts show that in *Astropecten scoparius* specimens smaller than about one cm in R bear poor diagnostic features.

DISCUSSION

The early development of astropectens in all six species so far reported is very similar in the following points: total equal cleavage, coeloblastula, and wrinkled blastula formation (Metschnikoff, 1885; Mortensen, 1921, 1937; Newth, 1925;

FIGURE 5. A) Skeletal system of metamorphosing bipinnaria, the same stage as shown in Figure 4G, H and I, view from the future oral side of the juvenile. Dotted line shows an outline of 5 hydrolobes: a, ambulacral plates; o, oral plates; scale = 40 μ. B) Metamorphosing bipinnaria with shrunken stalk, little later than that shown in Figure 2H, view from the future oral side: di, disk; st, stalk; scale = 100 μ. C) Opposite of the specimen shown in Figure 5B: c, central plate; di, disk; m, madreporic plate; st, stalk; t, terminal plate; scale = 100 μ. D) Metamorphosing juvenile, 16 days after insemination, view from the future oral side: o, oral plates; tf, tube-feet; tt, terminal tentacle; scale = 100 μ. E) Skeletal system of the oral side of the specimen shown in Figure 5D: a, ambulacral plates; o, oral plates; scale = 50 μ. F) Skeletal system of the aboral side of the specimen shown in Figures 5D and E: c, central plate; d, dorsal plates; m, madreporic plates; t, terminal plate; scale = 100 μ. G) Oral side of a juvenile immediately after the completion of metamorphosis, 18 days after insemination: e, eye-spot; mo, mouth; scale = 100 μ. H) Skeletal system of the juvenile shown in Figures 3A, B and 5G, aboral view: c, central plate; d, dorsal plates; m, madreporic plate; t, terminal plate; scale = 100 μ. I) Skeletal system of a ray of a juvenile shown in Figure 5G, oral view: a, ambulacral plate; o, oral plate; t, terminal plate; scale = 50 μ. J) Skeletal system of a ray of a juvenile, 5 days after the completion of metamorphosis, the same stage as shown in Figure 3D, oral view; scale = 100 μ. K) Skeletal system of the aboral side of a juvenile, 5 days after the completion of metamorphosis: c, central plate; m, madreporic plate; t, terminal plate; scale = 100 μ.

Hörstadius, 1939; Komatsu, 1973, 1975a; Oguro *et al.,* 1975). In the present study, it was found that the entire process of the development of *Astropecten scoparius* resembles that reported in *Astropecten aranciacus* (Hörstandius, 1939). They undergo metamorphosis while larvae are pelagic as bipinnaria, although there are some minor differences between them. Among those, the difference in the term of larval life seems to be noticeable. Although various factors such as temperature, food or population density could considerably affect the progress of development, a conspicuous difference found between the two species is unlikely due to an environmental cause (18 days in the present species versus 90 days in *Astropecten aranciacus* to complete metamorphosis). In *Astropecten aranciacus,* fully grown bipinnariae are furnished with well-developed bipinnaria arms and highly complex ciliary bands (Hörstadius, 1939; Figs. 22 and 23). On the other hand, the bipinnaria of the present species just before metamorphosis is fairly simple. The bipinnaria of *Astropecten scoparius* thus initiates metamorphosis prematurely in comparison to *Astropecten aranciacus,* with regard to the form of the bipinnaria. This may relate to the nutritional condition of the species. Eggs of *Astropecten scoparius* are larger and more lecithotropic than those of *Astropecten aranciacus,* in which a much longer term is needed to prepare for initiating metamorphosis. In this context, the development of *Astropecten latespinosus* is of special interest (Komatsu, 1975a). The eggs of *Astropecten latespinosus* is the largest of the three species, and metamorphosis is completed within five days after fertilization without feeding. Thus, the development of *Astropecten scoparius* lies between *Astropecten aranciacus* and *Astropecten latespinosus.*

The development of sea-stars is generally divided into two types, one called the direct type and the other, the indirect type. The former is found in species having yolky ova and develops through brachiolaria only. Observations on the entire developmental process of the direct type have been carried out in more than ten species. On the other hand, little is known of the development and metamorphosis in species having indirect development, due mainly to the difficulties in rearing planktonic larvae for long enough to initiate metamorphosis. Thus, the entire process of indirect development has so far been reported only for a few species.

The distinction between the two types is, however, not given clearly, as pointed out by Chia (1968) and Komatsu (1975a), although the terms have been habitually used. Attempts have been made by some workers, therefore, to give precise definitions for the two types of development. Development without free larvae was called the direct type by Hyman (1955). However, the free swimming brachiolaria of *Crossaster papposus, Certonardoa semiregularis* or of *Asterina coronata japonica* may be called free larvae, although their development is definitely of the direct type (Gemmill, 1920; Hayashi and Komatsu, 1971; Komatsu, 1975b). Chia (1968) then gave a more precise definition, that development with a larva having a functional larval gut should be called the indirect type, and that without a functional gut, the direct type. This distinction could be a good cue for dividing all asteroid larvae into two groups. However, in practice, the terms, "direct" or "indirect" are too sweeping. It is doubtful whether the term "indirect type" can adequately be applied to the development of *Astropecten aranciacus* or *Astropecten scoparius.* This is because although the "indirect type" indicates that these animals have bipinnaria with functional gut, it fails to signify that they do not pass through the brachiolaria stage.

An alternative proposition is here presented for distinguishing the characteristic development from usual indirect type of development. Development having brachiolaria only, irrespective of whether it is pelagic or benthic, is called the direct type. Development which passes through both the bipinnaria and the brachiolaria is called the indirect type. With this distinction, all developments of asteroids may be divided into two groups except a few species, including *Astropecten* species. As described before, development of all astropectens so far known passes through only the bipinnaria, or its equivalent, and never through the brachiolaria stage. This may be an important feature of the development of this group and should not be overlooked. In this regard, the term "nonbrachiolarian type" is tentatively proposed for the developmental type exemplified by *Astropecten* species. The following shows a scheme proposed here:

Type	Bipinnaria	Brachiolaria
indirect type	+	+
direct type	−	+
nonbrachiolarian type	+	−

Besides *Astropecten* species, only members of the genus *Luidia* undergo metamorphosis without passing through the brachiolaria stage (Mortensen, 1913, 1938). No other asteroids have been known to take the nonbrachiolarian type of development here defined.

In association with the facts mentioned above, it may be worthwhile to focus on the aboral skeletal plate formation. Immediately after the completion of metamorphosis, the aboral skeletal system of *Astropecten scoparius* is composed of one central plate, one madreporic plate and ten plates which correspond to radial and interradial plates. The same composition was reported in *Astropecten aranciacus* and *Astropecten latespinosus* (Hörstadius, 1939; Komatsu, 1975a). However, the sea-stars of the majority of species immediately after metamorphosis do not bear a madreporic plate. Only a few species have been known to develop the madreporic plate, which is independent of the interradial plate, at the time of formation of the primary aboral plates. Among the former are *Asterina gibbosa, Asterias rubens, Leptasterias aequalis, Pentaceraster mammillatus, Certonardoa semiregularis* and *Asterina coronata japonica* (MacBride, 1896; Gemmill, 1914; Gordon, 1929; Mortensen, 1938; Hayashi and Komatsu, 1971; Komatsu 1975b). It was reported as a remarkable case that in *Leptasterias ochotensis similispinis* the madreporite appears as a rudiment at the second year after metamorphosis (Kano, Komatsu and Oguro, 1974). *Luidia savignyi* is the only species so far reported to have a madreporic plate at metamorphosis, besides the *Astropecten* species (Mortensen, 1938). A similar fact was observed in *Luidia quinaria* in this laboratory (unpublished data). It is of special interest that early formation of the madreporic plate seems to be associated with the nonbrachiolarian type of development.

The fact that the appearance of both remarkable features is confined to the genera *Astropecten* and *Luidia,* which are designated as typical representatives of primitive asteroids (Fell, 1963; Heddle, 1967), may indicate that some developmental features in asteroids are in fact related to the systematic position of the species.

The writers wish to express their cordial thanks to Dr. Katsuma Dan, Professor Emeritus of Tokyo Metropolitan University, and to Dr. Ryoji Hayashi, Professor Emeritus of Toyama University, for their valuable suggestions and critical reading of the manuscript. Their thanks are extended to Professor Kazuo Suzuki, Messers Hideki Yoshizawa and Shoji Akabane, Matsumoto Dental College, for scanning electron microscopy. They are also indebted to Mr. Akira Sakashita, Directer of Uozu Aquarium, for his kind cooperation, especially for aquarium facilities.

The present study was in part supported by a Grant-in-Aid to CO and MK (No. 054012) and a Grant-in-Aid to MK (No. 074102) both from the Ministry of Education of Japan.

SUMMARY

1. *Astropecten scoparius* develops to a bipinnaria with simple ciliary bands and short bipinnaria arms through a wrinkled blastula by holoblastic, radial cleavage.

2. About seven days after insemination, the posterior portion of the bipinnaria becomes swollen and fine spicules appear on it, while the anterior portion, the stalk, remains unchanged.

3. Metamorphosis takes place gradually at the posterior portion, while the metamorphosing bipinnaria is pelagic. Two weeks after insemination, the stalk rapidly shrinks and the larva sinks to the bottom.

4. About 18 days after insemination, the juvenile completes metamorphosis with the opening of the mouth. The newly metamorphosed juvenile is 600 μ in diameter and each arm bears two pairs of the tube-feet, each having a sucker at the tip, and one terminal tentacle with red eye-spot.

5. The aboral skeletal system of the juvenile immediately after metamorphosis is composed of one central, one madreporic, ten radial and interradial plates, in addition to five terminal plates on the arms.

6. The juveniles smaller than about one cm in R do not bear some of the diagnostic features in this species.

7. A characteristic feature of the development of *Astropecten*, *i.e.*, the lack of a brachiolaria stage, is stressed. The term "nonbrachiolarian type" is tentatively proposed to distinguish the development of *Astropecten* and *Luidia*, which do not pass through a brachiolaria stage, from the usual indirect type of development.

LITERATURE CITED

ATWOOD, D. G., 1973. Larval development in the asteroid *Echinaster echinophorus*. *Biol. Bull.*, **144**: 1-11.

CHIA, F. S., 1968. The embryology of brooding starfish, *Leptasterias hexactis* (Stimpson). *Acta Zool.*, **49**: 321-364.

DAN, K., 1957. IX-3 Asteroidea. Pages 215-218 in M. Kume and K. Dan, Eds., *Invertebrate embryology*. Baifukan, Tokyo (in Japanese).

FELL, H. B., 1963. The phylogeny of sea-stars. *Phil. Trans. Roy. Soc. London Ser. B*, **246**: 381-435.

GEMMILL, J. F., 1914. The development and certain points in the adult structure of the starfish *Asterina rubens*, L. *Phil. Trans. Roy. Soc. London Ser. B,* **205**: 213–294.

GEMMILL, J. F., 1920. The development of the starfish *Crossaster papposus,* Müller and Troschel. *Quart. J. Microsc. Sci.,* **64**: 155–189.

GORDON, I., 1929. Skeletal development in *Arbacia, Echinarachnius* and *Leptasterias. Phil. Trans. Roy. Soc. London Ser. B,* **217**: 289-334.

HAYASHI, R., 1972. On the relations between the breeding habits and larval forms in asteroids, with remarks on the wrinkled blastula. *Proc. Jap. Soc. Syst. Zool.,* **8**: 42–48.

HAYASHI, R., 1973. *The sea-stars in Sagami Bay.* Biological Laboratory, Imperial Household, Tokyo, 114 pp.

HAYASHI, R. AND KOMATSU, M., 1971. On the development of the sea-star, *Certonardoa semiregularis* (Müller et Troschel) I. *Proc. Jap. Soc. Syst. Zool.,* **7**: 74–80.

HEDDLE, D., 1967. Versatility of movement and the origin of the asteroids. Pages 125-141 in N. Millott, Ed., *Echinoderm biology.* Academic Press, New York.

HENDERSON, J. A., AND J. S. LUCAS, 1971. Larval development and metamorphosis of *Acanthaster planci* (Asteroidea). *Nature,* **232**: 655–657.

HÖRSTADIUS, S., 1939. Über die Entwicklung von *Astropecten aranciacus* L. *Pubbl. Staz. Zool. Napoli,* **17**: 221–312.

HYMAN, L. H., 1955. *The Invertebrates, IV. Echinodermata.* McGraw-Hill, New York, 763 pp.

KANO, Y. T., M. KOMATSU AND C. OGURO, 1974. Notes on the development of the sea-star, *Leptasterias ochotensis similispinis,* with special reference to skeletal system. *Proc. Jap. Soc. Syst. Zool.,* **10**: 45-53.

KANATANI, H., 1969. Induction of spawning and oocyte maturation by l-methyladenine in starfishes. *Exp. Cell Res.,* **57**: 333–337.

KOMATSU, M., 1973. On the wrinkled blastula of the sea–star, *Astropecten scoparius. Zool. Mag.,* **82**: 204–207 (in Japanese with English abstract).

KOMATSU, M., 1975a. On the development of the sea-star, *Astropecten latespinosus* Meissner. *Biol. Bull.,* **148**: 49-59.

KOMATSU, M., 1975b. Development of the sea-star, *Asterina coronata japonica* Hayashi. *Proc. Jap. Soc. Syst. Zool.,* **11**: 42–48.

MACBRIDE, E. W., 1896. The development of *Asterina gibbosa. Quart. J. Microsc. Sci.,* **38**: 339–441.

MACBRIDE, E. W., 1914. *Text-book of embryology, Vol. I, Invertebrata.* Macmillan, London, 692 pp.

MASTERMAN, A. T., 1902. The early development of *Cribrella oculata* (Forbes), with remarks on echinoderm development. *Trans. Roy. Soc. Edinburgh,* **40**: 373-418.

METSCHNIKOFF, E., 1885. Über die Bildung der Wanderzellen bei Asterien und Echiniden. *Z. Wiss. Zool.,* **42**: 656–673.

MORTENSEN, T., 1913. On the development of some British echinoderms. *J. Mar. Biol. Ass. U.K.,* **10**: 1–18.

MORTENSEN, T., 1921. *Studies of the development and larval forms of echinoderms.* G.E.C. Gad., Copenhagen, 216 pp.

MORTENSEN, T., 1937. Contributions to the study of the development and larval forms of echinoderms. III. *Kgl. Dan. Vidensk. Selsk., Skr. Naturvid. Math. Afd. Ser. 9,* **7(1)**: 1-44.

MORTENSEN, T., 1938. Contributions to the study of the development and larval forms of echinoderms. IV. *Kgl. Dan. Vidensk. Selsk., Skr. Naturvid. Math. Afd. Ser. 9,* **7(3)**: 1–45.

NEWTH, H. G., 1925. The early development of *Astropecten irregularis,* with remarks on duplicity in echinoderm larvae. *Quart. J. Mirosc. Sci.,* **69**: 519-554.

OGURO, C., M. KOMATSU, AND Y. T. KANO, 1975. A note on the early development of *Astropecten polyacanthus* Müller et Troschel. *Proc. Jap. Soc. Syst. Zool.,* **11**: 49–52.

Reference: *Biol. Bull.* 151: 574–586. (December, 1976)

SYMBIOTIC ASSOCIATION OF *PHOTOBACTERIUM FISCHERI* WITH THE MARINE LUMINOUS FISH *MONOCENTRIS JAPONICA:* A MODEL OF SYMBIOSIS BASED ON BACTERIAL STUDIES

E. G. RUBY AND K. H. NEALSON

Scripps Institution of Oceanography, University of California, San Diego,
La Jolla, California 92093 U. S. A.

Mutually beneficial symbioses involving procaryotes and multicellular eucaryotes have been studied in systems such as the rumen (Gall and Huhtanen, 1951; Hungate, 1963), the root nodule of legumes (Allen and Allen, 1950), and the digestive tract of arthropods (Brooks, 1963; Fogelsong, Walker, Puffer and Markovetz, 1975). Such associates of bilateral benefit are often termed mutualisms (Henry, 1966).

The occurrence of luminous bacteria in specialized light-emitting organs of a variety of marine fishes is another example of procaryote/eucaryote mutualism. The fish provides the luminous bacteria within its light organ with a sheltered environment and a supply of nutrients and oxygen. The bacteria in turn serve as a continuous source of light which the fish uses for a variety of purposes (Harvey, 1952). The importance of this luminescence in the behavior of the flashlight fish, *Photoblepharon palpebratus*, has been described by Morin, Harrington, Kreiger, Baldwin and Hastings (1975) and McCosker and Lagios (1975). Although anatomical and histological morphology of the symbiotic light organs of a number of these fishes has been studied (Harvey, 1952; Ahrens, 1965), little is known of the biochemical interactions inherent in these interspecies associations.

Of the four species of luminous bacteria (Reichelt and Baumann, 1973), only two have been previously reported as symbionts in the light organs of fishes. In this report a third species, *Photobacterium fischeri* is identified as the bacterial component of the light organ of the Japanese pinecone fish, *Monocentris japonica.* Thus, for the first time a symbiotic niche has been found for this species. A representative bacterial isolate from the light organ is characterized with regard to physiological parameters of its light emitting system and a speculative model of the symbiosis discussed.

MATERIALS AND METHODS

Bacterial strains and media

Luminous bacteria from the light organ of *Monocentris japonica* were isolated as described below. Additional strains used were *Photobacterium fischeri* (B-398) and *Beneckea harveyi* (B-392), a strain previously designated *P. fischeri* MAV (Nealson and Markovitz, 1970; Nealson, Eberhard and Hastings, 1972). The strain numbers refer to those assigned by Reichelt and Baumann (1973). The generic assignment of some species is not yet agreed upon. Hendrie, Hodgkiss and Shewan (1970) proposed four groups: *Photobacterium phosphoreum, P. mandapamensis, Vibrio fischeri,* and *Lucibacterium harveyi.* Reichelt and Baumann (1973, 1975), whose assignments we follow, referred to these groups as *P. phosphoreum, P. leiognathi, P. fischeri,* and *Beneckea harveyi,* respectively.

The sea water media used in this study were prepared with artificial sea water (ASW) consisting of 0.4 M NaCl, 0.1 M $MgSO_4$ $7H_2O$, 0.02 M KCl, and 0.02 M $CaCl_2$ $2H_2O$ (MacLeod, 1968). The basal medium broth (BM) contained 50 mM Tris-HCl (pH 7.5), 19 mM NH_4Cl, 0.33 mM K_2HPO_4 $3H_2O$, 0.01 mM $FeSO_4$ and half-strength ASW (MacLeod, 1968). Basal medium agar (BMA) was prepared by separately sterilizing and then mixing equal volumes of double-strength BM and 20 g of Difco Noble Agar per liter. Compounds serving as sole sources of carbon and energy were filter-sterilized (0.2 μ Nucleopore) and added to the already autoclaved medium. For a complex medium broth (LM), or agar (LMA) 5 g Bacto-Peptone and 3 g Difco Yeast Extract and 3 ml glycerol were added to the recipe for BM. All media used in experiments dealing with acid production were modified by replacement of Tris buffer with 50 mM Hepes (pH 7.5) and by the exclusion of glycerol. Various sugars were added as indicated.

Living specimens of the Japanese pinecone fish, *Monocentris japonica,* were collected in the summer of 1975, fifty miles southeast of Tokyo and shipped alive to the Steinhart Aquarium. Light organs from four fish (A–D) were used. Bacterial isolation (A) was the only one performed on a healthy living fish. The other three isolations (B, C and D) were made from intact light organs of fish that had been dead for less than 12 hours prior to sampling. Otherwise the procedure was the same for the four isolations. The lower jaw containing the pair of anterio-lateral light organs was placed on ice and the surface of the organ and surrounding tissue was swabbed with 75% ethanol. The organ was then slit with a sterile razor blade and a sterile micropipette inserted into the organ matrix from which about 2–5 μl of organ fluid could be removed. This fluid was diluted into sterile sea water by a factor of 5×10^6 and several 0.1 ml aliquots of the diluted fluid were spread on LMA plates which were incubated at 18° C.

Methods

Taxonomic identification of the luminous bacterial isolates was accomplished using criteria established by Reichelt and Baumann (1973). The method involves a determination of nutritional versatility on minimal medium (BMA) with one of twelve compounds as sole source of carbon and energy. In addition, the production of three extra-cellular enzymes was monitored as well as the ability to grow at 35° C on LMA. These sixteen characteristics are diagnostic for the four species of luminous bacteria, *Beneckea harveyi, Photobacterium fischeri, P. phosphoreum,* and *P. leiognathi* (Reichelt and Baumann, 1973). It should be noted here that the species of *Photobacterium* referred to as *P. mandapamensis* by Reichelt and Baumann (1973) has been named, on the basis of priority as *P. leiognathi* (Reichelt and Baumann, 1975). The table also contains data concerning the production of a yellow pigment, and the type of decay kinetics of *in vitro* luciferase assays (Hastings and Mitchell, 1971). These additional tests have recently been added to the diagnostic taxonomy of the luminous bacteria (J. Reichelt, personal communication).

Growth of batch cultures was monitored both by optical density at 660 nm in a Coleman Jr. II Spectrophotometer, and by electronic counting using a Coulter ZBI Particle Counter. An optical density value of 0.1 units is equivalent to 2.7×10^8 cells/ml for a range of 0.05–0.5 optical density units.

Cells in liquid culture were monitored for light production utilizing an EMI Type 9781A phototube and Pacific Photometrics model 110 amplifier with an Esterline Angus Servo Speed recorder. Periodically a 0.1 ml sample of the culture was removed to a clean glass scintillation vial. The vial was placed in a light-tight chamber and exposed to the phototube to measure the level of *in vivo* luminescence. The output of the photometer was expressed in light units, where one light unit was determined to be 2×10^{10} quanta/sec by the radioactive standard of Hastings and Weber (1963).

Autoinducer (Nealson, Platt and Hastings, 1970), which accumulates in the culture medium, was prepared by growing a representative strain of *Monocentris* symbiont (MJ1) in BM to an optical density value of 0.8 (3.5×10^9 cells/ml). Cells were removed from the spent growth medium by centrifugation and the supernatant fraction was sterilized by filtration (Nucleopore, 0.2 μ). The autoinducer fraction could then be frozen until assayed. A cross reaction was prepared to determine how addition of the autoinducer preparation affected the onset of bioluminescence in strains MJ1, and B-398. Cells of these strains were innoculated to a low optical density (0.01) in 20 ml of BM. Five ml were dispensed to two growth tubes, and 0.1 ml of the autoinducer preparation added to one tube. The second tube was a control receiving 0.1 ml of BM. The tubes were shaken at 150 rpm and 23° C, and growth and luminescence monitored at 15 minute intervals. Sensitivity of a strain to the presence of the autoinducer compound present in the spent medium of MJ1 was indicated by a significantly earlier onset of induction of luminescence in tubes with added inducer, compared to the control tubes (Eberhard, 1971).

The glucose concentration in cell-free medium was determined by the glucose oxidase reaction using the Glucostat method (Worthington Biochemical). Pyruvate was assayed in a cuvette containing 2 ml of 50 mM Tris buffer (pH 7.5), 0.2 ml of 10 mM NADH and 0.1 ml of medium sample. The absorbance at 340 nm was measured using a Beckman DU spectrophotometer and the decrease in absorbance after addition of 2 units of LDH (0.1 ml) was recorded (Lowry and Passonneau, 1972). This value was compared to a standard curve using known concentrations of pyruvic acid.

Cells were innoclulated into 250 ml flasks containing 150, 100, or 50 ml of LM. The flasks were placed on a New Brunswick G24 Environmental Incubator rotary shaker at 100 rpm and 21° C. Because of the gentle shaking, oxygen diffuses more slowly into medium in the flask with the lower surface to volume ratio (150 ml) than into the flask with 100 ml, which in turn is slower than the flask with 50 ml. All of the cultures have a characteristic aerobic growth rate up to an optical density of 0.15 to 0.25. After this point the growth rate is limited to an extent dependent on the degree of oxygen availability. Thus the effect of oxygen limitation on the development of the luminescence system can be ascertained.

Tubes containing 5 ml of modified LM were innoculated with log-phase cells of MJ1 to a concentration of 10^7 cells per ml. Sterile solutions of either glucose, mannitol, glycerol, galactose or mannose were added to pairs of tubes to give a 0.2% solution and the cultures grown at 22° C and 200 rpm. At an optical density of 0.3 (4×10^8 cells/ml) the cells were harvested by centrifugation (13,000 *g* for 15 min). Organic acids in the supernatant were detected by the gas-liquid chromatographic method of the Virginia Polytechnical Institute Anaerobic Laboratory (1973).

MJ1 cells were grown in 35 ml of modified LM plus 0.2% glucose by shaking at 150 rpm in a 200 ml flask at 22° C. At an optical density of 0.46 (1.2×10^9 cells/ml) the medium was centrifuged (10,000 *g* for 10 min) and the supernatant discarded. The pellet was resuspended in 5 ml of ice-cold, sterile sea water and recentrifuged. After carefully drawing off the supernatant, 5 ml of modified BM plus 0.2% glucose was added to the tube and the pellet rapidly resuspended. One ml was distributed to each of 5 small (10 ml capacity) centrifuge tubes which were immediately placed on a shaker at 300 rpm and 22° C. At 0, 5, 10, 15 and 20 minutes, a tube was removed from the shaker and plunged

TABLE I

Results of the taxonomic characterization of 48 bacterial isolates obtained from the luminous organs of four fish (A, B, C and D) compared to the phenotype of the standard strain of Photobacterium fischeri *(B-398). Columns summarize all phenotypes observed, plus (+) denoting presence of trait, and minus (−) denoting absence. Kinetics of* in vitro *luciferase assay were fast-type (F) or slow-type (S). (P.f. represents* Photobacterium fischeri; *B. sp.,* Beneckea *species.)*

Number of isolates from each fish	*Photobacterium fischeri* (B-398)	Fish						
		A 29		*B* 5		*C* 5	*D* 9	
Tests								
Growth on:								
Cellobiose	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+
d-Xylose	−	−	−	−	−	−	−	−
Mannitol	+	+	+	−	−	+	−	+
d-Gluconate	−	−	−	−	−	−	−	+
d-Glucuronate	−	−	−	−	−	−	−	+
*d*L-Lactate	−	−	−	−	−	−	−	+
Acetate	+	+	+	+	−	−	+	+
Pyruvate	−	+	+	+	+	+	+	+
Propionate	−	−	−	−	−	−	−	+
d-α Alanine	−	−	−	−	−	−	−	+
L-Proline	+	+	+	−	−	+	+	+
Extracellular production of:								
Amylase	−	−	−	−	−	+	−	+
Gelatinase	−	−	+	−	−	−	−	+
Lipase	+	+	+	+	+	+	+	+
Growth at 35°C	±	+	+	−	−	−	−	+
Produce yellow pigment	+	+	+	+	+	+	+	−
Luciferase kinetics	F	F	F	F	F	F	F	S
Number of isolates with phenotype		27	2	4	1	5	8	1
Taxonomic identity	*P.f.*	*P.f.*	*P.f.*	*P.f.*	*P.f.*	*P.f.*	*P.f.*	*B. sp.*

into ice water to suspend cellular activity. The tubes were centrifuged and the supernatant examined for glucose and pyruvate by enzyme assays.

RESULTS

Isolation and taxonomy

The light organ of *Monocentris japonica* contains symbiotic bacteria that are located extracellularly in parallel tubular ducts whose possible communication with the exterior has not been described. Electron microscopy reveals these tubules to be densely packed with bacteria of a single morphological type. It is thus not surprising that cell densities of 5.6 and 9.4×10^9 bacteria per ml of organ fluid were determined from LMA plate counts of two such organs. Primary isolation of several hundred bacteria from light organs of four fish revealed that 100% of the colony forming units were luminous.

Five to twenty-nine colonies were chosen at random as representatives of the populations of four organs of four separate fish, subjected to taxonomic analysis and compared to previously identified strains (Table I). Three important results were obtained from this work: first, with the exception of a single isolate from fish "D" all isolates were of the species *Photobacterium fischeri;* secondly, isolates from each organ were predominantly of one phenotype; and thirdly, the phenotypes of bacteria from organs of different fish differed by several traits and no two were the same. It is important to note that the degree of variation of any of the six phenotypes from that of the standard strain is well within limits of positive identification as *P. fischeri.*

Bacterial physiology

It was of interest to examine in batch culture the response of *in vivo* bioluminescence of MJ1 cells to physiological conditions of the medium. A major factor which controls the development of luminescence is autoinducer (Nealson, Platt and Hastings, 1970; Eberhard, 1971), which is produced by luminous bacteria, excreted into the medium during growth and, upon reaching a critical concentration, induces the synthesis of luciferase. Addition of spent medium of the *Monocentris* symbiont (MJ1) stimulates the induction of *in vivo* light emission of cells both of its own strain and of another strain of *Photobacterium fischeri* (B-398) (Fig. 1). This effect has been shown to be due to the presence of large amounts of autoinducer in the spent medium of MJ1. As previously reported there was no cross reaction between species (Magner, Eberhard and Nealson, 1972). No effect of the MJ1 autoinducer on the light emission of cells of *Beneckea harveyi* was observed, nor did MJ1 cells induce luminescence sooner with the addition of spent medium of *B. harveyi.*

Because oxygen is a substrate for the luminescence reaction, its effect on the development of the light emitting system was examined. It can be seen that the amount of light produced per unit cell material increases when cells are grown in lower ambient oxygen concentrations (see Materials and Methods). That is, more synthesis of the luminous system occurs in cells grown in lower oxygen concentrations (Fig. 2).

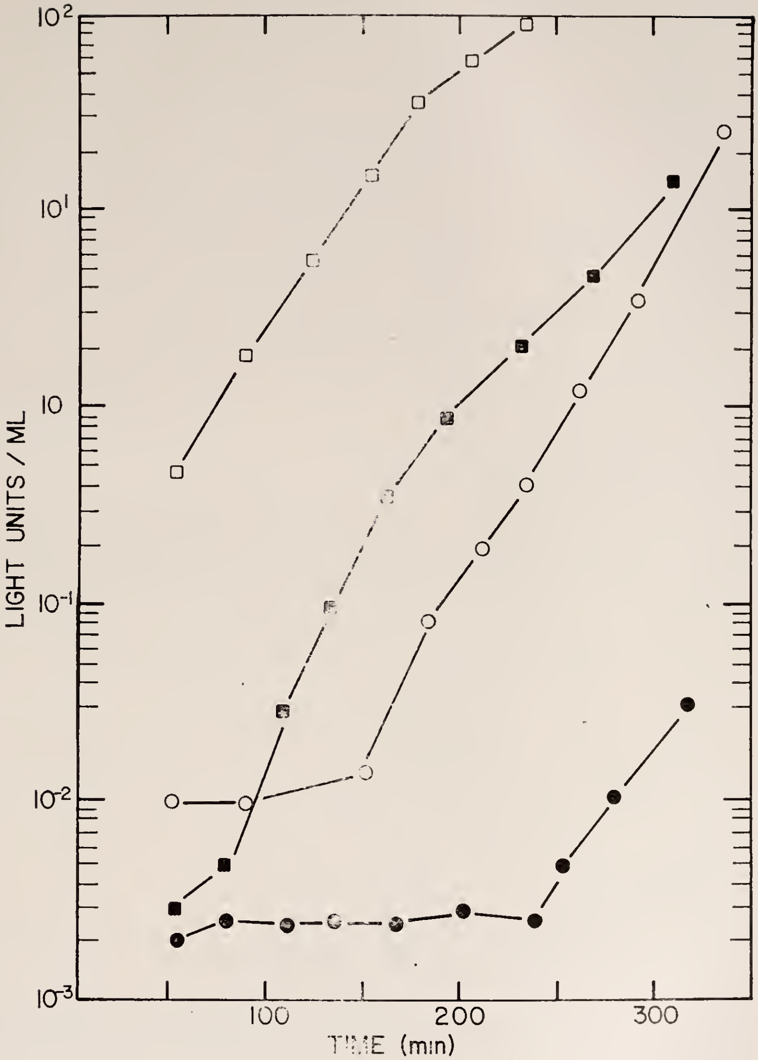


FIGURE 1. Effect of autoinducer from strain MJ1 on the induction of bioluminescence of MJ1 (open square) and B-398 (closed square). Control cultures of MJ1 (open circle) and B-398 (closed circle) received no added inducer. There was no difference in the growth rates of cultures with or without added inducer.

In addition to the factors mentioned above, the nature of the substrate utilized during growth also plays a role in the control of luciferase production. If brightly luminescing (induced) cultures of MJ1 are diluted to an optical density below 0.05 (1.4×10^8 cells/ml), the *in vivo* light of the culture will not increase until the cells have reached a certain density in the medium and luminescence is induced. If the cells are grown in a glycerol medium and diluted into fresh medium con-

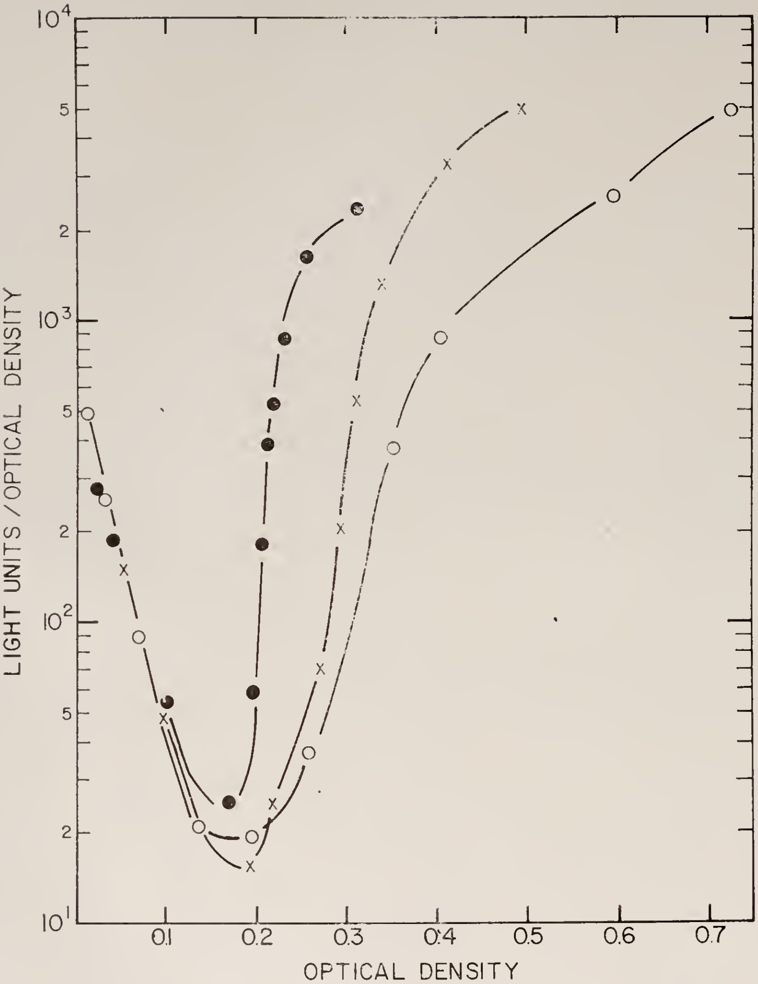


FIGURE 2. Specific activity of *in vivo* light production by MJ1 during growth at different oxygen concentrations (achieved by using different volumes of medium). Symbols designate flasks with 50 ml (open circle), 100 ml (X), or 150 ml (closed circle) of medium per flask. High values of bioluminescence per cell are reached earliest in the culture with the most volume, which is most limited for oxygen (closed circle), and latest in the culture least limited (open circle).

taining glucose, the point at which induction occurs is delayed relative to that in cells diluted into fresh glycerol medium (Fig. 3A). However, cells pregrown several generations on glucose will not experience that increased lag period when again diluted into glucose medium (Fig. 3B).

Further examination of this glucose effect reveals that glucose addition to cells growing on glycerol will either delay induction or, if induction has already begun, cause a temporary suspension of it (Fig. 4). This glucose effect is neither reversed by cAMP (cyclic adenoside monophosphate) nor caused by the glucose analogue 2-deoxy-glucose.

During aerobic growth of MJ1 on glucose, the pH of the medium drops to below 5.0 at cell densities above 5×10^8 cells/ml and luminescence is then extinguished. Addition of strong buffers (Tris or Hepes) delays or reverses this effect, indicating that it is probably due to acid production.

To determine the identity of the acid(s) responsible for the decrease in pH of the medium, MJ1 cells were grown in a complete medium with glucose to an optical density of 0.3 (9×10^8 cells/ml). Cells were removed by centrifugation and a chromatographic analysis was performed on extracts of the medium. Of the acids detectable by gas-liquid chromatography (formate, pyruvate, lactate, oxalacetate, and succinate), pyruvate was the principal compound present in the spent medium, sometimes reaching concentrations of several millimolar. In addition, pyruvate concentration is a direct function of cell number. Growth on galactose, mannose, glycerol or mannitol, however, results in less than 5% of the acid levels obtained with glucose.

To determine what percentage of the glucose utilized by the cells was being converted to pyruvate cultures growing in a complete medium with glucose were transferred to minimal medium with glucose. Both the utilization of glucose and the excretion of pyruvate were then monitored for twenty minutes (Fig. 5). Pyruvate accounted for 30–40% of the glucose-carbon metabolized based on the

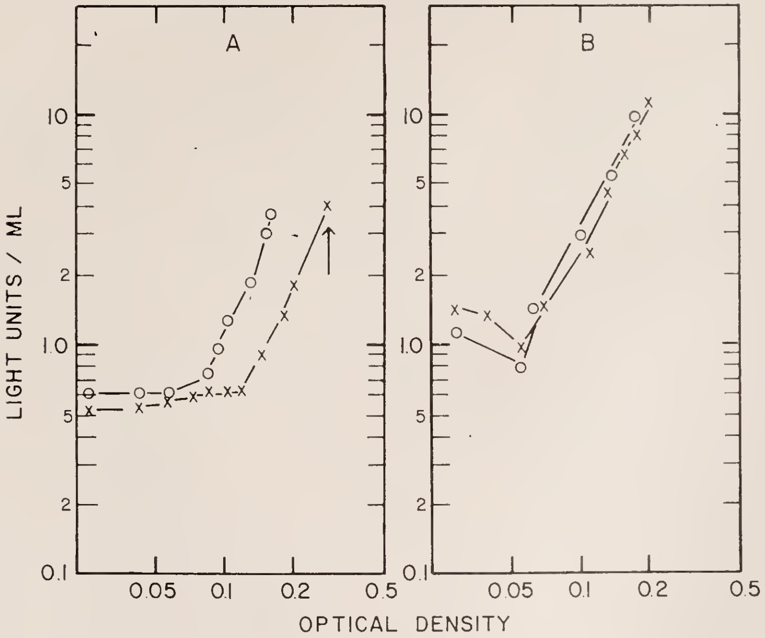


FIGURE 3. A. Development of *in vivo* luminescence in MJ1 cells pregrown in glycerol medium and innoculated into fresh medium with either glycerol (open circle) or glucose (X) as the energy source. B. Cells removed from glucose culture at arrow and innoculated into fresh medium with either glycerol (open circle) or glucose (X).

calculation [millimoles of pyruvate produced/2 (millimoles of glucose utilized)] × 100%. It should be noted that MJ1 cells are capable of metabolizing pyruvate as evidenced by their ability to grow on pyruvate as the sole source of carbon and energy (Table I).

DISCUSSION

Although luminous bacteria are known to be the source of light for many luminous marine fishes, these bacteria have been isolated from other habitats, including the surfaces of decaying marine organisms and directly from sea water (Harvey, 1952). All four bacterial species (*Beneckea harveyi, Photobacterium fischeri, P. phosphoreum,* and *P. leiognathi*) have been isolated both directly from sea water or as saphophytes; however, only members of the genus *Photobacterium* have been found in symbiotic association. Such findings have led to a descriptive designation of *Beneckea harveyi* as "free-living" and the other genus of luminous

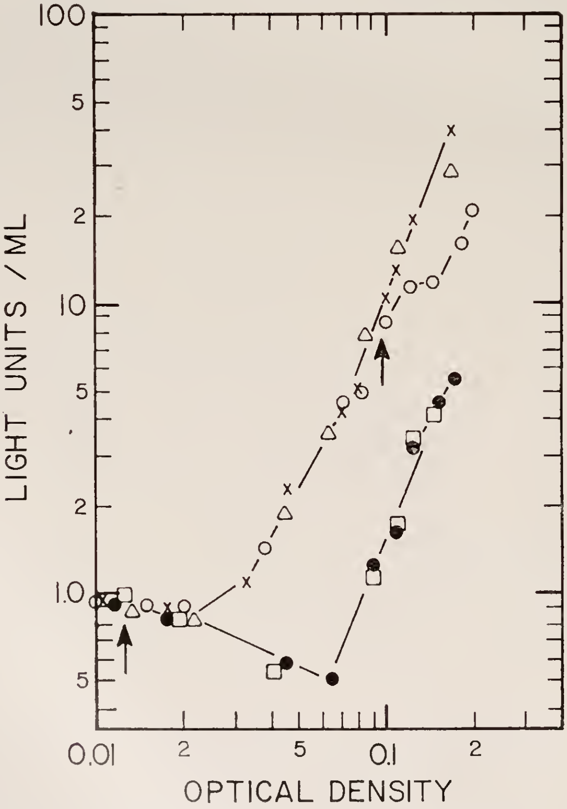


FIGURE 4. Effect of the addition of glucose on *in vivo* induction of the luminous system of MJ1: glycerol control (no glucose) (X); 0.2% glucose added at first arrow (closed circle); 0.2% glucose plus 0.3 mg/ml cAMP added at first arrow (open square); 0.2% glucose added at second arrow (open circle); and 0.2% 2-deoxyglucose at second arrow (open triangle.)

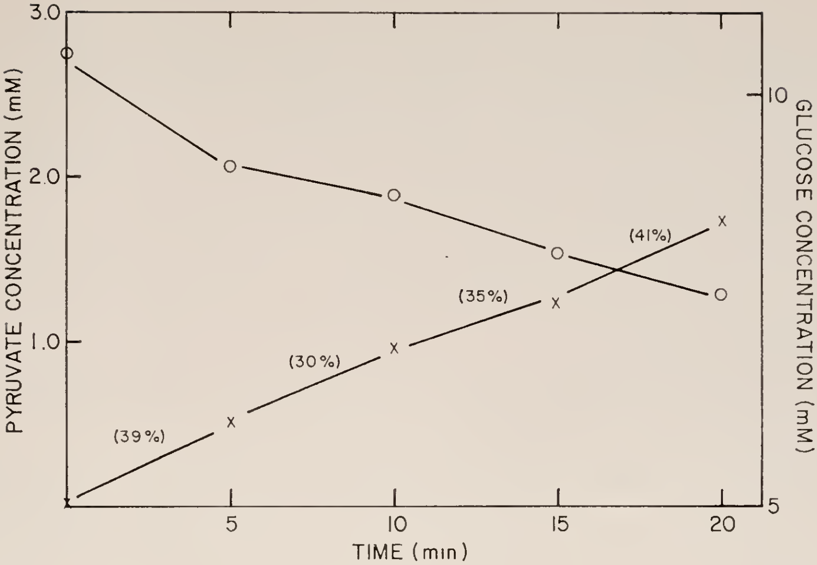


FIGURE 5. Appearance of excreted pyruvate (X) and removal of glucose (open circle) during short term exposure of strain MJ1 to minimal medium plus glucose (3.5×10^9 cell/ml). Numbers in parentheses indicate the percentage of glucose-carbon utilized that appears in the medium as pyruvate.

bacteria, *Photobacterium,* as "symbiotic" (Hastings and Mitchell, 1971). However, of the three species of *Photobacterium,* only *P. leiognathi* (Boisvert, Chatelain and Bassot, 1967; Reichelt and Baumann, 1975) and *P. phosphoreum* (Herring, 1975) have been found associated with light organs of luminous fishes. No report of a symbiotic niche for *P. fischeri* has yet been put forth. In this study we have shown such an association of *P. fischeri* with the luminous fish *M. japonica.*

Yasaki (1928) first established the bacterial origin of the luminosity of the Japanese pinecone fish *Monocentris japonica.* More recently Graham, Paxton and Cho (1972) characterized 13 luminous bacteria isolated from the Australian pinecone fish, *Cleidopus gloriamaris.* Although some of these were certainly *P. fischeri,* it was not reported which of the isolates were obtained directly from the organ and which were from the mouth and body surfaces of the fish. Luminous strains from the light organs of a number of other species of fishes have also been isolated and studied (Harvey, 1952; Boisvert *et al.,* 1967; Yoshiba and Haneda, 1967; Hastings and Mitchell, 1971). Although it was known from these studies that light organs contain luminous bacteria that appear similar both microscopically and in colonial morphology, these involved no taxonomic characterization. In order to understand the symbiotic relationship between fish and microbe, the identity of the bacterial component of the association must be known. In the present study bacteria were isolated only from the interior of the light organs and, of the 48 isolates from four fish, all but one clearly belong to the species *Photobacterium fischeri.* Similar findings for isolates of *P. leiognathi* from several leiognathid fishes will be reported elsewhere (Nealson, Reichelt and Hastings, in preparation).

The way in which the luminous organ obtains its bacterial innoculum is not known; nor is the degree of isolation of the organ culture from exchange and/or contamination with the external environment known. Clearly there must be host mechanisms involving selection for symbiont characters, but as yet we have no knowledge as to the specific features and mechanisms which operate.

The several physiological properties of MJ1 described in this study may be considered in terms of their suitability for adaptation to symbiosis. For example, the occurrence of autoinduction could provide a way for the bacteria to enjoy two different "life styles": symbiotic and free living. In bacteria confined within the luminous organ, the autoinducer of luciferase could accumulate and stimulate the synthesis of the bioluminescence system; whereas while free in sea water, no such accumulation would occur.

The enhancement of the synthesis of the luminescent system at low oxygen levels might also be related to the symbiotic relationship. The maintenance of a low ambient oxygen concentration in the light organ would produce maximal luminescence with a minimal commitment to bacterial growth.

It is difficult to envision a metabolic purpose for harvesting only a small amount of the energy available in glucose and excreting pyruvate, a compound which the cell is quite capable of catabolizing further. It is known, however, that excretion of metabolites is a characteristic phenomenon of symbioses in general (Buchner, 1965; Smith, Muscatine and Lewis, 1969). There are numerous examples of regulatory and nutritional communication between symbiotic partners by means of excretion of large amounts of carbon compounds (Muscatine, Karakashian and Karakashian, 1967; Muscatine, Boyle and Smith, 1974).

In *B. harveyi* the induction of the synthesis of the luminescent system is repressible by glucose, this being reversible by cAMP (Nealson, Eberhard and Hastings, 1972). In MJ1 there is no such catabolite repression of bioluminescence, as defined by Ullman and Monod (1968) and Tyler and Magasanik (1970). Although glucose exerts a temporary inhibition upon the development of luminescence, it is not reversible by cAMP, nor does it occur with the analogue, 2-deoxyglucose. Once adapted to growth on glucose, MJ1 cells can produce an amount of light equal to that obtained with glycerol. Thus, in the symbiotic light organ, *Monocentris* could supply its bacterial culture with glucose as a substrate and still achieve a high efficiency of light production with *P. fischeri* (MJ1) as the symbiont, but not with *B. harveyi.*

The blood of marine fishes contains glucose as its principal nutrient (Dittmer, 1961; Umminger, 1970); at the concentrations reported (0.07 to 0.15%), glucose could readily support the metabolic demands of these bacteria, based on studies with the isolated cells. Glucose utilization would lead to the excretion of pyruvic acid and unless this metabolite were removed, the pH within the organ would drop and extinguish the bioluminescence. We can speculate that the fish tissue surrounding the bacteria could absorb the pyruvate and metabolize it aerobically using the numerous large mitochondria located in the cells adjacent to the bacterial culture. The utilization of oxygen in this process could serve to poise its concentration at the level optimal for luciferase synthesis. Such a model could account for a system by which the fish regulates the oxygen concentration within the organ to maintain a population of slowly growing, brightly luminescing bacteria.

SUMMARY

Isolation of bacteria from the luminous organ of the fish *Monocentris japonica* has revealed that the organ contains a pure culture of luminous bacteria. For the four fish examined, all contained *Photobacterium fischeri* as their luminous bacterial symbiont. This is the first time that *P. fischeri* has been identified in a symbiotic association.

A representative isolate (MJ1) of the light organ population was selected for *in vivo* studies of its luminous system. Several physiological features suggest adaptation for symbiotic existence. First, MJ1 has been shown to produce and respond to an inducer of luciferase that could accumulate in the light organ. Secondly, the specific activity of light production was seen to be maximal under low, growth-limiting concentrations of oxygen. Thirdly, unlike another luminous species (*Beneckea harveyi*), synthesis of the light production system of these bacteria is not catabolite repressed by glucose—a possible source of nutrition in the light organ. Fourthly, when grown aerobically on glucose these bacteria excrete pyruvic acid into the medium. This production of pyruvate is a major process, accounting for 30–40% of the glucose utilized and may serve as a form of regulatory and nutritional communication with the host.

LITERATURE CITED

AHRENS, G., 1965. Untersuchungen am Leuchtorgan von *Leiognathus Klunzingeri* (Steindachner). *Z. Wiss. Zool.*, **173**: 90–113.

ALLEN, O. N. AND E. K. ALLEN, 1950. Biochemical and symbiotic properties of the rhizobia. *Bacteriol. Rev.*, **14**: 273–330.

BOISVERT, H., R. CHATELAIN AND J.-M. BASSOT, 1967. Étude d'un *Photobacterium* isolé de l'organe lumineux de poissons Leignathidae. *Ann. Inst. Pasteur*, **112**: 520–524.

BROOKS, M. A., 1963. Symbiosis and aposymbiosis in arthropods. Pages 200–231 in P. S. Nutman and B. Mosse, Eds. *Symbiotic associations.* Cambridge University Press, New York and London.

BUCHNER, P., 1965. *Endosymbiosis of animals with plant microorganisms.* Interscience Publishers, New York, 909 pp.

DITTMER, D., 1961. Editor, *Blood and other body fluids.* Federation of American Societies for Experimental Biology, Washington, D. C., 540 pp.

EBERHARD, A., 1971. Inhibition and activation of bacterial luciferase synthesis. *J. Bacteriol.* **109**: 1101–1105.

FOGLESONG, M. A., D. H. WALKER, JR., J. S. PUFFER AND A. J. MARKOVETZ, 1975. Ultrastructural morphology of some prokaryotic microorganisms associated with the hindgut of cockroaches. *J. Bacteriol.*, **123**: 336–345.

GALL, L. S. AND C. N. HUHTANEN, 1951. Criteria for judging a true rumen organism and a description of five rumen bacteria. *J. Dairy Sci.*, **34**: 353–362.

GRAHAM, P. H., J. R. PAXTON AND K. Y. CHO, 1972. Characterization of luminous bacteria from the light organs of the Australian pinecone fish (*Cleidopus gloriamaris*). *Arch. Mikrobiol.*, **81**: 305–308.

HARVEY, E. N., 1952. *Bioluminescence.* Academic Press, New York, 649 pp.

HASTINGS, J. W., AND G. W. MITCHELL, 1971. Endosymbiotic bioluminescent bacteria from the light organ of the pony fish. *Biol. Bull.*, **141**: 261–268.

HASTINGS, J. W. AND K. WEBER, 1963. Total quantum flux of isotopic sources. *J. Opt. Soc. Amer.*, **53**: 1410–1415.

HENDRIE, M. S., W. HODGKISS AND J. SHEWAN, 1970. The identification, taxonomy and classification of luminous bacteria. *J. Gen. Microsc.*, **64**: 157–169.

HENRY, S. M., 1966. *Symbiosis, Vol. I.* Academic Press, New York, 477 pp.

HERRING, P. J., 1975. Bacterial bioluminescence in some argentanoid fishes. *Proc. European Mar. Biol. Symp.*, **9**: 563–572.

Hungate, R. E., 1963. The rumen bacteria. Pages 266-297 in P. S. Nutman and B. Mosse, Eds., *Symbiotic associations.* Cambridge University Press, New York and London.

Lowry, O. H. and J. V. Passonneau, 1972. *A flexible system of enzymatic analysis.* Academic Press, New York, 291 pp.

MacLeod, R. A., 1968. On the role of inorganic ions in the physiology of marine bacteria. *Advan. Microbiol. Sea,* **1**: 95-126.

Magner, J., A. Eberhard and K. Nealson, 1972. Characterization of bioluminescent bacteria by studies of their inducers of luciferase synthesis. *Biol. Bull.,* **143**: 469.

McCosker, J. E., and M. D. Lagios, 1975. Les petits peugeots de Grand Comore. *Pacific Discovery,* **28**: 1-6.

Morin, J. G., A. Harrington, K. Nealson, N. Krieger, T. O. Baldwin and J. W. Hastings, 1975. Light for all reasons: versatility in the behavioral repertoire of the flashlight fish. *Science,* **190**: 74-76.

Muscatine, L., J. E. Boyle and D. C. Smith, 1974. Symbiosis of the acoel flatworm *Convoluta roscoffensis* with the alga *Platymonas convolutae. Proc. Roy. Soc. London Ser. B.,* **187**: 221-234.

Muscatine, L., S. J. Karakashian nd M. W. Karakashian, 1967. Soluble extracellular products of algae symbiotic with a ciliate, a sponge and a mutant hydra. *Comp. Biochem. Physiol.,* **20**: 1-12.

Nealson, K. H. and A. Markovitz, 1970. Mutant analysis and enzyme subunit complementation in bacterial bioluminescence in *Photobacterium fischeri. J. Bacteriol.,* **104**: 300-312.

Nealson, K. H., A. Eberhard and J. W. Hastings, 1972. Catabolite repression of bacterial bioluminescence: functional implications. *Proc. Nat. Acad. Sci. U. S. A.,* **69**: 1073-1076.

Nealson, K. H., T. Platt and J. W. Hastings, 1970. Cellular control of the synthesis and activity of the bacterial luminescence system. *J. Bacteriol.,* **104**: 313 322.

Reichelt, J. L. and P. Baumann, 1973. Taxonomy of the marine, luminous bacteria. *Arch. Mikrobiol.,* **94**: 283-330.

Reichelt, J. L. and P. Baumann, 1975. *Photobacterium mandapamensis* Hendrie *et al.,* a later subjective synonym of *Photobacterium leiognathi* Boisvert *et al. J. Syst. Bacteriol.,* **25**: 208-209.

Smith, D., L. Muscatine and D. Lewis, 1969. Carbohydrate movement from autotrophs to heterotrophs in parasitic and mutualistic symbiosis. *Biol. Rev.,* **44**: 17-90.

Tyler, B. and B. Magasanik, 1970. Physiological basis of transient repression of catabolite enzymes in *Echerichia coli. J. Bacteriol.,* **120**: 411-422.

Ullman, A. and J. Monod, 1968. Cyclic AMP as an antagonist of catabolite repression in *Escherichia coli. FEBS Letters,* **2**: 57-60.

Umminger, B., 1970. Physiological studies of supercooled killifish (*Fundulus heteroclitus*); III. Carbohydrate metabolism and survival at subzero temperatures. *J. Exp. Zool.,* **173**: 159-174.

Virginia Polytechnic Institute, 1973. *Anaerobic laboratory manual, 2nd edition.* Southern Printing, Blacksburg, Virginia, 114 pp.

Yasaki, Y., 1928. On the nature of the luminescence of the knight-fish, *Monocentris japonicus* (Houttuyn). *J. Exp. Zool.,* **50**: 495-505.

Yoshiba, S. and Y. Haneda, 1967. Bacteriological study on the symbiotic luminous bacteria cultivated from the luminous organ of the apogonid fish, *Siphamia versicolor* and the Australian pinecone fish, *Cleidopus gloria-maris. Sci. Rep. Yokosuka City Mus.,* **13**: 82-85.

Reference: *Biol. Bull.* **151**: 587–600. (December, 1976)

EXCHANGES OF SODIUM AND CHLORIDE AT LOW SALINITIES BY *NEREIS DIVERSICOLOR* (ANNELIDA, POLYCHAETA)

RALPH I. SMITH[1]

Visiting Investigator, Zoophysiological Laboratory A, August Krogh Institute,
University of Copenhagen, 2100 Copenhagen Ø, Denmark

Recent studies on the uptake and exchanges of water and the principal inorganic ions in the well-known brackish-water polychaete *Nereis diversicolor* (F. O. Müller) indicate that this worm employs a number of physiological mechanisms in its osmotic and ionic regulation (Oglesby, 1970, 1972; Smith 1970a, b, c; Fletcher, 1970, 1974a, b, c; review by Oglesby, 1969a), but there remain many unanswered questions regarding the inter-relationships of these processes and of their control. One area of interest is that of the relationship between the regulation of the two major ions, sodium and chloride. Smith (1970a, b, c) as well as Jørgensen and Dales (1957) and Oglesby (1969b) has postulated an active uptake of chloride, without specifying whether chloride as such is being transported, or moved as a consequence of or in relation to the transport of some other ion, such as sodium. Jørgensen and Dales postulated reductions in permeability of the body wall both to water and chloride, but Smith (1970a, b) demonstrated a reduction in apparent permeability to water without finding it necessary to invoke a reduction in permeability to chloride. Jørgensen and Dales suggested that the urine of *N. diversicolor* could be iso-ionic in respect to chloride; Smith (1970c) demonstrated hypo-osmotic urine. Oglesby (1970, 1972) has provided much detailed evidence on the steady-state levels of water, chloride, sodium and potassium, and especially on the efflux of sodium; he has found an active uptake of sodium as well as a reduction in body-wall permeability to it at low salinities and gave evidence that potassium is not regulated in the body fluid. Fletcher (1970) studied the relationship of the inside-negative body wall potential to external salinity, as well as the regulation of calcium and magnesium, and the control of body volume (1974a, b, c).

The present study includes a comparison of the patterns of sodium and chloride exchange rates as functions of salinity in the steady state and an attempt to determine whether or not the sodium uptake of *N. diversicolor* is activated at low salinities. The assumptions and conclusions involved in the chloride balance-sheet presented by Smith (1970a, b) are reinvestigated and are modified in the light of fresh data.

MATERIALS AND METHODS

Basic methods

Nereis diversicolor was collected in shallow water of Vellerup Vig, a small bay of the Danish Isefjord, at a site where salinity is markedly, although variably, lowered by inflow of a freshwater stream. This site was the source of worms used by Smith (1955a), Jørgensen and Dales (1957), and Ahearn and Gomme (1975). The biology of *N. diversicolor* in the Isefjord has been discussed in detail by Rasmussen (1973). After collection, worms were transported to Copenhagen in undiluted Isefjord water (*ca.* 65 per cent sea water, % SW), and sorted in the laboratory into large plastic boxes provided with numerous short lengths of glass tubing, in which worms took up residence within a few hours. Worms were maintained with aeration at 14° C in various dilutions of artificial sea water made up according to Hale (1958). "100% SW" as used in these studies had a sodium concentration of 470 mM/liter and a chloride concentration of 550 mM/liter. It was diluted with distilled water for concentrations down to 5% SW. Below 5% SW, dilutions were with a solution resembling hard pond water (Smith, 1970a), but lacking Na and Cl. This dilutant contained Ca^{++}, 1.14 mM liter; Mg^{++}, 0.39 mM/liter; K^+, 0.11 mM/liter, and was made up at 10× final strength. K was added as $KHCO_3$ or KNO_3, Mg as $MgSO_4$, and Ca as $Ca(OH)_2$; the cloudy alkaline fluid resulting was cleared by *ca.* 25 drops of conc. H_2SO_4 per liter to a final pH of 5.5 or 6. The product could not be told by taste from distilled water, and permitted good survival in SW dilutions down to 0.2% SW (ca. 0.9 mM Cl/liter). There was survival in 0.1% SW so diluted but, as the worms were noticeably sluggish and were sticky from excess mucus, they were not used experimentally. For simplicity, dilutions of sea water are expressed in the text as the percentage of sea water (% SW), since a given dilution of SW has different molarities of Na and Cl.

---

[1] Author's address: Department of Zoology, University of California, Berkeley, California, 94720 U. S. A.

Adaptations to various salinities were made stepwise over periods of one to several days, and worms remained at the final adaptational salinity for 4–10 days before being used in experiments. In the standard pattern of experiment, used for most ^{22}Na and all ^{36}Cl exchange studies, worms were exposed individually for 1 hour in 10 ml of radioactive medium at 15° C. In any single experiment 16 worms were used, and sub-groups of 5–8 worms were tested at different salinities at the same time, these groupings being arranged so as to cancel out progressive or serial changes resulting from different lengths of residence under laboratory conditions which might impose some trend upon the results of studies made on successive days.

^{22}Na-uptake. ^{22}Na was obtained from the Radiochemical Centre, Amersham, England as neutral NaCl. Media were measured into vials in a water-bath at 15° C. For each experiment, worms were visually sorted into approximately equal size groups, weighed after blotting on filter paper, and dropped individually into a series of vials of 15 ml of adaptational medium at 15° C, this medium being the same as that for the radioactive tracer to be used next. This passage through an intermediate inactive medium served to provide a period of adjustment and a comparable activation for all worms, since it is known (Smith, 1970a) that worms so transferred between identical media initially show a net loss of chloride and of weight as a result of the expulsion of urine with the increased activity. Starting 30–60 minutes after the transfer to adaptational medium, worms were blotted and transferred at 2-minute intervals to 10 ml of medium containing the tracer, still at 15° C. After one hour of undisturbed exposure, worms were individually blotted, rinsed in a fresh sample of inactive medium for 2–4 seconds, re-blotted,

and dropped into 2 ml of 4% formaldehyde previously measured into a series of polyethylene counting vials and tightly stoppered. Samples were counted to a constant 10,000 counts on a "Selektronic" solid state (NaI) scintillation gamma counter with sample changer and printout. Four 10 μl portions of unused radioactive medium of each salinity employed, as well as a number of blanks for background, were also counted in each experiment.

^{36}*Cl-uptake.* ^{36}Cl was obtained as neutral NaCl from the Danish Atomic Energy Commission Research Center, Risø, Denmark. Worms were handled as above, up to the point of removal from the one-hour exposure in radioactive medium, blotting, rinsing, and re-blotting. In ^{36}Cl-exchange measurements, worms were then dropped individually into a previously measured 2 ml of 3 N HNO_3 in tightly-capped resistant polyethylene centrifuge tubes and digested for 48 hr at room temperature, with two or three vigorous mechanical shakings to ensure complete disruption, centrifuged, and a 1 ml sample of clear supernatant transferred to a capped plastic scintillation vial, to which 10 ml of scintillation fluid was later added (one liter toluol, DDH, sulfur-free, Merck; 550 ml Triton-X; 5 g PPO; 200 mg POPOP). One ml of the digesting acid was added to each of a number of vials for background counts, and 10 μl of each active medium was added to one ml of acid, in triplicate, for determination of its activity.

Sodium concentration. Sodium was measured by means of an Eppendorf Flame Photometer (Netheler and Hinz GMBH, Hamburg), employing NaCl standards diluted 1 : 500 in 1.5 mM/liter KNO_3 to counteract potassium interference. Samples of media, coelomic fluid, and the acid ^{36}Cl extracts were diluted in this medium also, usually 1 : 500, although with media of 1% SW, 50 μl were added to 5 ml, and of 0.2% SW, 100 μl had to be used.

Chloride concentration. Chloride was measured by use of a model CMT10 Chloride Titrator (Radiometer, Copenhagen), employing NaCl standards from the same stock used for sodium, but diluted 1 : 100 in the same KNO_3 dilutant. Appropriate dilutions were made of media, coelomic fluid, and the acid ^{36}Cl extracts, namely, 1 : 100 for coelomic fluid samples and for media of 20% SW and stronger, 1 : 20 for 10% SW, and full strength for media of 5% SW or lower concentration. In the latter instances, the volume titrated could be varied by use of appropriate Petersen constriction pipettes.

Experimental procedures

Pattern of steady-state sodium exchange as a function of salinity. The pattern of steady-state sodium exchange in *Nereis diversicolor* has been thoroughly investigated by Oglesby (1970, 1972) in studies focused upon sodium efflux in worms from an estuarine habitat in northern England. In the present study of worms from the more stable intermediate salinities of the Isefjord, comparative data were obtained; these will be seen to be in line with Oglesby's results, although exact correspondence was neither obtained nor expected. In the initial series of experiments, worms were exposed for different lengths of time (0.5 to 4 hr) to radioactive media containing ^{22}Na, and interpolation was used to obtain a rate of exchange for one hour. This method proved cumbersome, so that a standard one-hour exposure was adopted in subsequent work with ^{22}Na and for all work with ^{36}Cl.

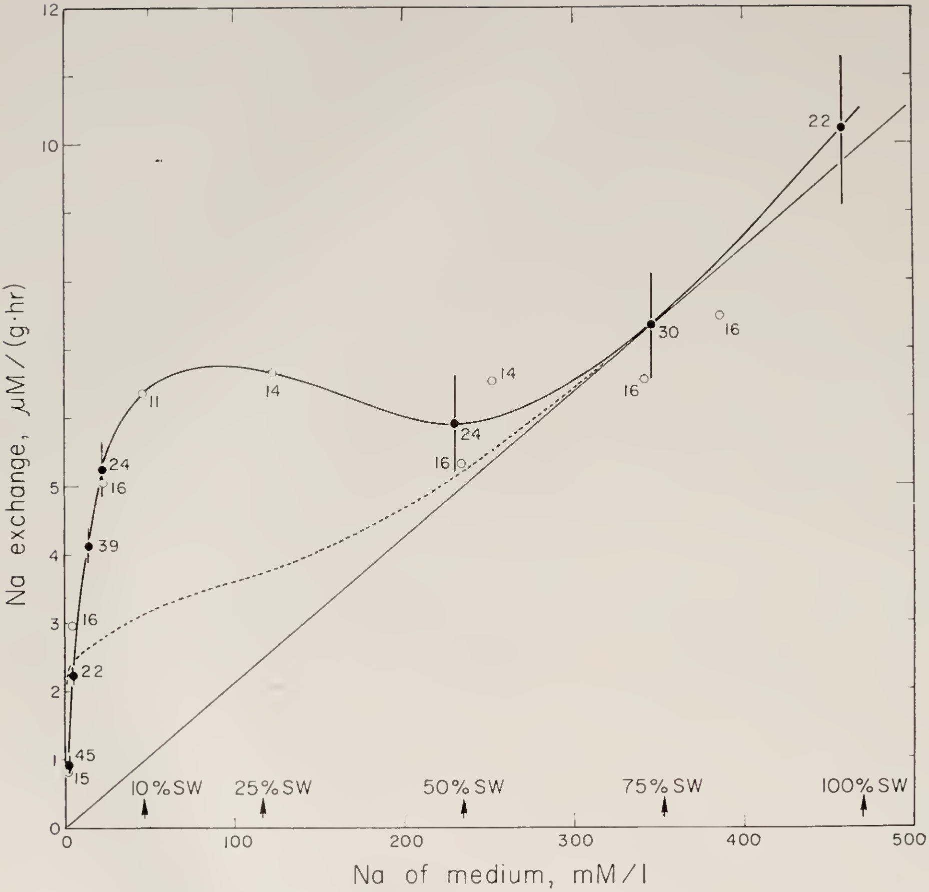


FIGURE 1. Steady-state Na-exchange in *N. diversicolor* (as influx of ^{22}Na into whole body) as a function of external concentration. Closed circles and solid curve calculated from one-hour exposures; open circles by interpolation to one hour; number of worms (n) is shown by each point. In this and following figures, vertical bars show ± 2 s.e. Dotted curve shows passive integumental diffusional efflux, proportional to coelomic Na-concentration (determined on 98 worms), calculated on the assumption that Na-permeability of body wall does not change with salinity. Diagonal straight line shows passive diffusional influx, proportional to external Na-concentration, also assuming no change in Na-permeability, and assuming that total exchange in 75% SW is passive.

Pattern of steady-state chloride exchange as a function of salinity. The rate of Cl-uptake or steady-state exchange in *N. diversicolor* as a function of the external chloride concentration has been shown by Jørgensen and Dales (1957) and Smith (1970a) to have a characteristic pattern with the exchange proportional to external chloride concentration in salinities greater than 50% SW, a more or less constant level over the intermediate range of salinities, a tendency to show a peak of exchange in low salinities (± 10% SW), and a marked reduction in very low salinities (down to 0.4% SW, = *ca.* 2.5 mM Cl/liter). Smith (1970a) constructed a balance-sheet for chloride exchanges, using the assumptions of Potts and

Parry (1964), by which it was possible to conclude that *N. diversicolor* could maintain chloride balance at an external chloride concentration as low as 2.5 mM/liter by utilizing the observed rate of Cl-uptake and the presumed active recovery of chloride from the consequently hypotonic urine, *without the necessity of postulating a reduction in the permeability of the body wall to chloride,* as had Jørgensen and Dales. The inferences drawn from the balance-sheet were supported by the demonstration (Smith, 1970b) of a reduction in the apparent permeability to water of the body wall (resulting in the reduction of urine volume), and by the direct demonstration (Smith, 1970c) that the urine of *N. diversicolor* is hypo-osmotic to coelomic fluid at low salinities.

However, the chloride balance-sheet used by Potts and Parry (1964) and by Smith (1970a) involves acceptance of two questionable assumptions, namely, that the electrical potential across the body wall may be ignored, and that the permeability to diffusional (passive) exchange of chloride does not change with salinity. But in view of the evidence for such a change presented by Jørgensen and Dales (1957), and the fact that Oglesby (1970) demonstrated a reduction in the permeability of the body wall to sodium (confirmed in the present study), it is necessary to re-examine the assumption of no chloride-permeability change. That Smith (1970a) found it unnecessary to invoke such a permeability change was because, in his study, the total Cl-uptake (= total efflux) was, at all salinities down to 0.4% SW, greater than the calculated passive or diffusional integumental output of chloride. The purpose of the present work was to re-study chloride uptake at even lower salinities, in order to test whether or not a Cl-permeability change must be invoked. Secondly, the intention was to compare the patterns of sodium and chloride exchange at low salinities to see if there are indications that the course of Cl-uptake is independent of Na-uptake, or is in some way coupled to or dependent upon Na-uptake.

Test for activation of Na-uptake mechanism at low salinities. It has been shown in gammarid crustaceans (Shaw and Sutcliffe, 1961) that adaptation to very low environmental Na-concentrations results in an activation of the sodium uptake mechanism. This was shown by the fact that, when gammarids adapted to a very low sodium concentration were transferred to a higher concentration, the rate of uptake of sodium exceeded that which would be expected of animals in steady-state adaptation to the higher concentration. It is of interest to determine if a comparable response is shown by *Nereis diversicolor.* Worms were adapted for five or more days at 15° C to salinities of *ca.* 0.2, 1, 3, and 5% SW (Na-concentrations of *ca.* 1.1, 4.6, 13.6, and 22.3 mM/liter, respectively). Uptake of ^{22}Na was then measured in a one-hour exposure, in the following groupings:

$$\begin{tikzcd}
\text{Adapted in low Na} \arrow[r] \arrow[dr] & \text{tested in low Na} \\
 & \text{tested in higher Na} \\
\text{Adapted in higher Na} \arrow[r] & \text{tested in higher Na}
\end{tikzcd}$$

In any single experiment, involving 16 worms, groups of 5–6 individuals representing all three transfer classes were tested simultaneously in order to compensate for any serial or progressive change in behavior resulting from increasingly long periods of laboratory adaptation, or from factors which might vary from day to day. The uptakes recorded in symmetrical tranfers (adaptational and test media the same) formed the basis for the "steady state" uptake curve in Figure 4, with which the results of asymmetrical transfer (from a low to a higher Na-concentration) are compared. "Low" Na-concentrations were 0.2 and 1% SW; "higher" concentrations were 1, 3, and 5% SW in these experiments. An activation of the Na-uptake mechanism should result in the "transfer" curve lying above the "steady-state" curve, if other factors remained the same.

RESULTS

Pattern of steady-state sodium exchange as a function of salinity

As shown in Figure 1, the results of interpolation to one hour were in general agreement with the values for total Na-influx obtained for one-hour exposures upon which the uptake curve is drawn. This curve shows several characteristics of the steady-state exchange of sodium (in the steady state the total Na-influx equals the total Na-efflux). First, at higher salinities (75–100% SW) the exchange of sodium is proportional to external and internal (coelomic fluid) Na-concentrations, as would be expected in the range of ionic conformity if no active transport were taking place. However, in the 50–75% SW range, where ionic

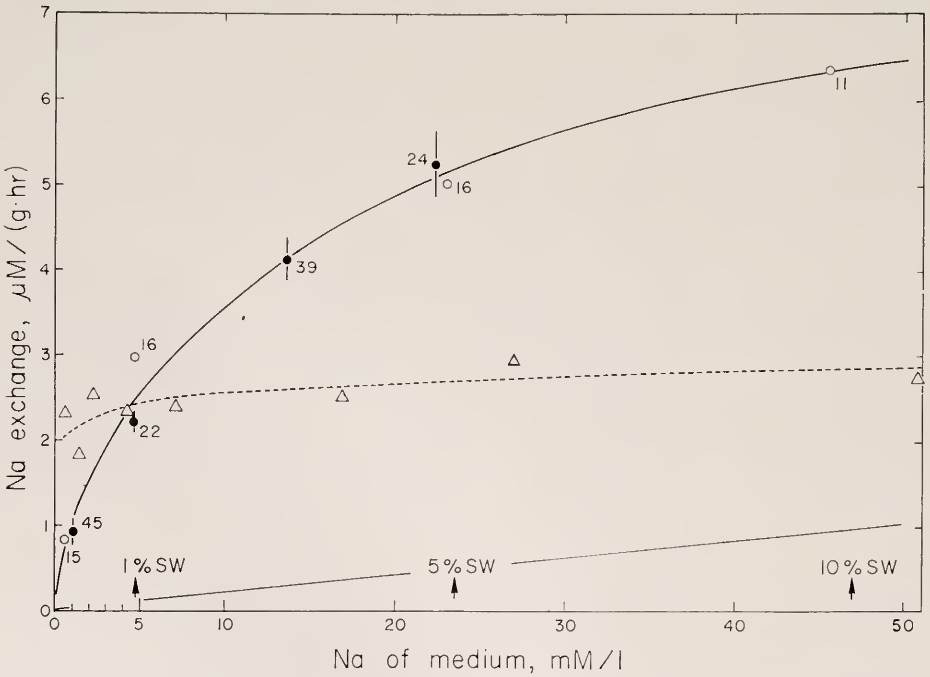


FIGURE 2. Expansion of Na-exchange curves of Figure 1 for Na–concentration range below 50 mM/liter. Symbols as in Figure 1, with addition of triangles representing passive diffusional efflux values, calculated from coelomic fluid Na-concentrations, on which dotted passive efflux curve is based.

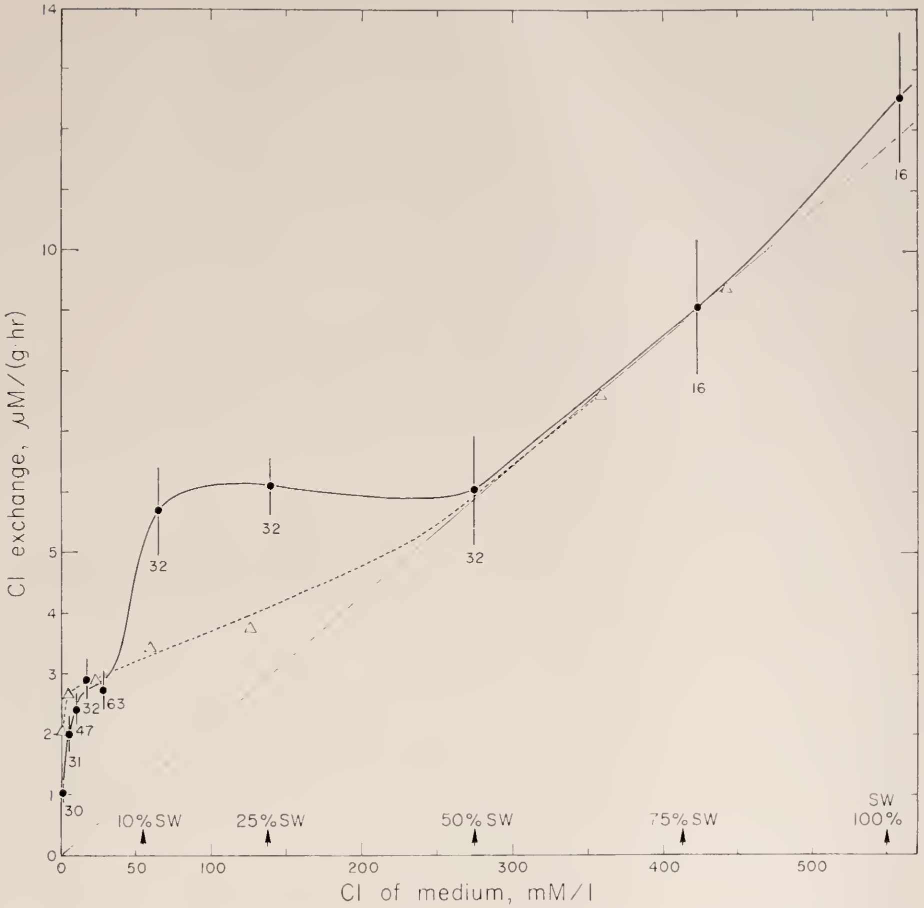


FIGURE 3. Steady-state Cl-exchange in *N. diversicolor* (as influx of ^{36}Cl into whole body) as a function of external concentration. Closed circles and solid curve indicate Cl-influx; triangles and dotted curve show integumental diffusional efflux, proportional to coelomic Cl-concentration (determined on 101 worms), calculated on assumption of no change in Cl-permeability of body wall. Diagonal straight line shows passive diffusional influx, proportional to external Cl-concentration, also assuming no change in Cl-permeability, and assuming that total exchange in 75% SW is passive.

conformity also prevails, the exchange is somewhat elevated, suggesting some active transport process. In Figures 1 and 2 the straight diagonal line represents the *passive influx* that would be expected on the basis of external Na-concentration alone, assuming no change in Na-permeability of the body wall. The dotted curve in Figures 1 and 2 represents the *passive efflux* expected on the basis of internal (coelomic) Na-concentration alone, again assuming no change in body-wall Na-permeability. Secondly, below 50% SW, the measured rate of Na exchange (equal to total influx in the steady state) is much elevated above the level of passive efflux, indicating nondiffusional (urinary) loss of sodium, as well as active inward transport process(es). There is a tendency for Na-exchange to be maximal in *ca.* 20% SW or below, where urinary output and inward sodium transport are presumed to be high. Thirdly, there is a very marked exchange at low external Na-concentrations; if a value of *ca.* 6.5 μM Na/(g·hr) represents the saturation level of the system, then the external concentration necessary for half the maximum uptake rate is *ca.* 10 mM/liter. The exchange rate rises rapidly with increased availability of sodium, to level off at 6–7 μM Na/(g·hr). But at the lowest external Na-concentrations, below ca. 6 mM/liter, the measured Na-exchange falls below the diffusional efflux of sodium that would be calculated if Na-permeability were constant at all salinities (Figs. 1 and 2).

Pattern of steady-state chloride exchange as a function of salinity

Chloride influx in the present experiments has the pattern shown in Figure 3. As in the earlier study (Smith, 1970a), there is proportionality of uptake to Cl-concentration in the range of 50–100% SW, where coelomic and external Cl-concentrations are nearly equal. Over the range from *ca.* 10 to 50% SW the influx is fairly constant, and lies well above the calculated integumental diffusional efflux (dotted curve), suggesting that in this range of ionic and osmotic regulation there must be a considerable nonintegumentary (probably urinary) loss of chloride as well as active inward transport of chloride. The peak of Cl-exchange indicated in *ca.* 10% SW by Jørgensen and Dales (1957) and by Smith (1970a) is not evident in the present data but, it may be noted, such a peak was not obtained in the previous shorter-term (4 hr) experiments (Smith, 1970a). Below 10% SW, the uptake curve drops markedly, and clearly lies below the dotted curve of integumental diffusional output in the salinity range from 5% SW down to *ca.* 0.2% SW [Cl. *ca.* 1 mM/liter; half the lowest value tested by Smith (1970a)]. In this low-salinity range, the chloride-exchange curve is depressed below that for sodium (Fig. 1), leveling off in the range from 2 to 5% SW and then resuming its rise to a plateau level of *ca.* 6 μM Cl/g·hr). This level of chloride uptake, measured as uptake of the whole body, is consistent with the uptake plateau of *ca.* 8 μM/(g·hr) recorded by Smith (1970a) in the shorter-term (4 hr) exposures, calculated on the basis of activities obtained in coelomic fluid. Such determinations were misleadingly high, and resulted from the oversimplification of regarding *N. diversicolor* as a single compartment of coelomic fluid surrounded by a body wall. The present results differ most markedly from the earlier results in that the total Cl-exchange in the salinity range below 8–10% SW is too low to maintain the steady state in the face of a diffusional integumental efflux calculated on the assumption of no change in diffusional permeability to chloride, hence the latter assumption is untenable. Further, the depression of the chloride exchange curve in the 1–5% SW range is in sharp contrast to the very steep and uninterrupted rise of the sodium exchange curve in this low salinity range (Fig. 1).

Test for activation of Na-uptake mechanism at low salinities

The results of these experiments are in general insufficient to support the hypothesis of an activation of the sodium uptake mechanism of *N. diversicolor*

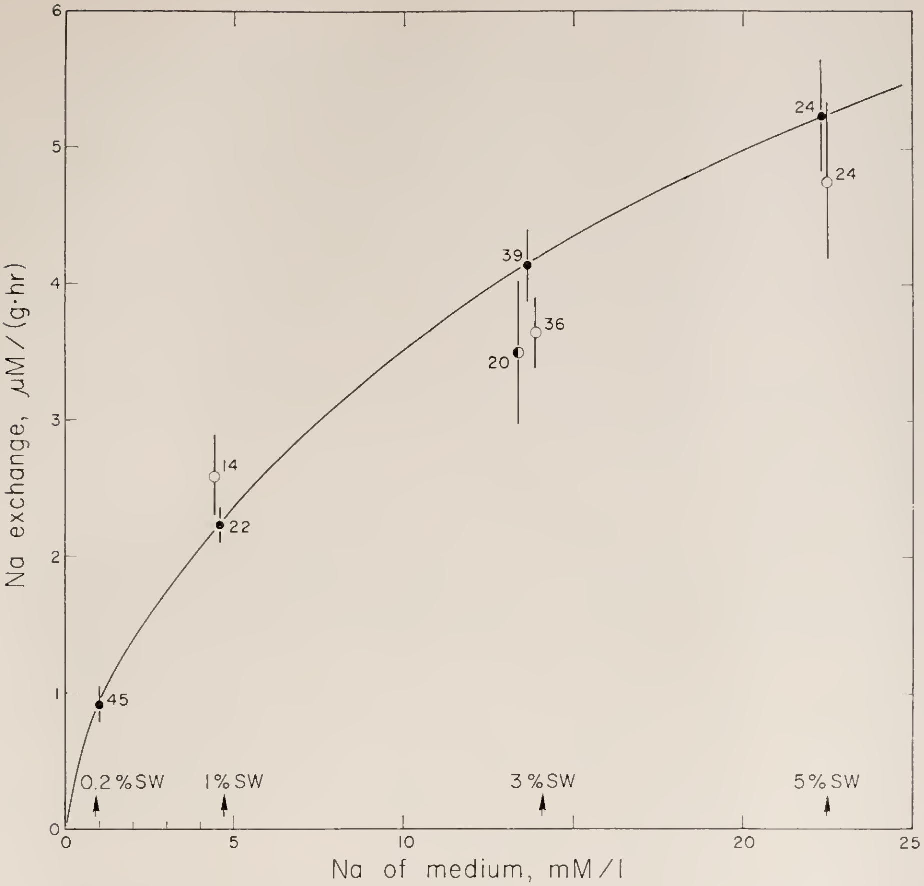


FIGURE 4. Effect upon Na exchange-rate of adaptation to very low Na-concentrations followed by abrupt transfer to higher concentrations. Closed circles and curve indicate Na-influx of control worms in steady state adaptation; open circles show Na-influx after transfer from 0.2% SW (Na = *ca.* 1 mM/liter), half-open circle after transfer from 1% SW (Na = *ca.* 5 mM/liter).

at low salinities. As is indicated in Figure 4, only in the case of transfer from 0.2% SW to 1% SW is there a scarcely significant increase of Na-uptake over the rate characteristic of worms adapted to 1% SW. In all other types of transfer: from 0.2% SW to 3% or 5% SW, and from 1% to 3% SW, the uptake as measured in the first hour after transfer tended to fall below the steady-state curve. Although the rates of the several transfer groups are not statistically significantly lower, they are generally below the steady-state curve and (with the possible exception of the 0.2 to 1% SW transfer) indicate no activation of Na-uptake. The combined results of transfer from 0.2 and 1% SW to 3% SW are significantly lower statistically than the steady-state uptake rate of animals adapted to 3% SW.

Discussion

The pattern of sodium-exchange as a function of external concentration (Fig. 1) suggests that the uptake mechanism for sodium operates with a high affinity for that ion, down to external concentrations of 1 mM/liter or less, at which the uptake rate is very low [< 1 μM/(g·hr)]. It must follow, as Oglesby (1972) has already shown, that *N. diversicolor,* in adapting itself to salinities of 1–2% SW, must utilize a lowering of body wall permeability to sodium as part of its adaptational repertoire. Otherwise, outward diffusion of Na from the high concentration maintained in the body fluid would exceed the measured steady-state influx. The dotted curve in Figure 1, shown in more detail in Figure 2, represents the integumental diffusional efflux calculated *on the assumption* that the permeability to sodium does not change and that outward diffusion of sodium is proportional to the concentration of Na in coelomic or extracellular fluids. At external Na-concentrations below *ca.* 5 mM/liter (*ca.* 1% SW), the sodium influx could not balance the efflux, hence the assumption of a constant diffusional sodium-permeability cannot be supported. In contrast to the pattern of Cl-exchange (Fig. 3), there is little indication of proportionality of Na-exchange to external salinity in the 50–75% SW range as might be expected *on the assumption* that only passive diffusional exchange takes place in the range of osmotic and ionic conformity, and by analogy with the evidence for chloride. The straight line in Figure 1, drawn on the assumption that exchange is proportional to external Na-concentration in 75% SW and higher salinities, lies sufficiently below the Na-exchange curve as to suggest that it is not necessary to assume that the active uptake mechanism for sodium is simply shut off when a state of conformity is reached. The present results suggest, rather, that some degree of active inward transport of sodium, with some form of coupled outward transport (possibly exchange diffusion) continues to operate in the 50–75% SW range in steady-state conformity. Oglesby, however, (1972) found exchange diffusion of Na only in salinities below 25% SW, and the matter needs further examination.

The pattern of chloride exchange (Fig. 3) differs in certain details, both from the earlier results obtained by Smith (1970a), and from the pattern of sodium exchange obtained in the present study (Fig. 1), so as to indicate that the active inward transport of sodium and chloride are by independent processes. Chloride exchange rates as determined in England by Smith (1970a) were based upon ^{36}Cl-activity measured in coelomic fluid, and in consequence appear higher than the values obtained in the present study of uptake by whole worms (in which the intracellular compartment, low in chloride, is included). In Smith's (1970a) studies, *N. diversicolor* was treated as a single compartment of coelomic fluid within a body wall, the Cl-permeability of which was assumed to be unchanged at lower salinities. The calculation of an integumental diffusional efflux *on the assumption that outward diffusional permeability remained constant* was done in order to permit discussion of the treatment of water and chloride fluxes in *N. diversicolor* in the terms used by Potts and Parry (1964, pp. 145–152). The resulting balance sheet for chloride fluxes proved useful at the time, but it is now apparent that it was based on certain untenable assumptions, and must be set aside. The assumption that the outward diffusional permeability to chloride is constant is discredited by the present results because, as in the case of sodium, the uptake rate of chloride at very low salinities in these experiments proves inadequate to maintain the steady state without reduction of the calculated diffusional efflux, shown by the dotted curve in Figure 3. It is no longer possible, by such a simple balance-sheet as was used by Smith (1970a) to fractionate the steady-state Cl-efflux between an "integumental" and a "urinary" component. It can be concluded that *N. diversicolor,* in maintaining chloride balance at very low salinities, does utilize Cl-permeability reduction, as postulated by Jørgensen and Dales (1957), together with a reduction of apparent water-permeability and the active inward transport of chloride both from the medium and from the consequently hypotonic urine. The previous neat and simple balance sheet is, however, inadequate to depict the chloride exchanges at low salinities, and must, in that respect at least, be discarded.

The pattern of Cl-exchange (Fig. 3) further differs from the previous (Smith, 1970a) exchange curve for this ion, as well as from the Na-exchange curve (Fig. 1), in showing a depression in the Cl-concentration range below 50 mM/liter (*ca.* 10% SW). At least four hypotheses to account for such a depression might be considered. First, there might be a physiological difference between *N. diversicolor* populations of British estuaries and those of the more stable intermediate salinities of the Danish Isefjord. There is no factual basis for this hypothesis; such evidence as exists from studies employing identical methods on British and Danish *N. diversicolor* (Smith, 1955b) suggest similarity rather than differences in the regulation of chloride. Secondly, there might be some as-yet-undetected technical or experimental flaw. This is a possibility difficult to prove or disprove. The 1970a study employed planchet counting of ^{36}Cl in coelomic fluid samples after 4 and 18 hour exposures; the present study used scintillation counting of acid extracts of whole worms after one hour exposures, but there is no reason why such differences in method should alter the shape of the Cl-exchange curve. After discounting the above hypotheses, two physiological hypotheses may be considered.

The first of these is that there might be two different chloride uptake mechanisms involved. One, operating at very low Cl-concentrations, might have a high affinity for chloride and a low capacity for transport; it would have a concentration for half-maximal rate of uptake of about 4 mM Cl/liter and would be essentially saturated at an external Cl-concentration of *ca.* 20 mM/liter, with a maximal uptake rate of *ca.* 3 μM Cl/(g·hr). The second, operating over a wider salinity range, would have lower affinity but a higher capacity for chloride; it would be saturated at an external Cl-concentration of *ca.* 75 mM/liter, with an uptake rate approaching 6 μm Cl/(g·hr), and would attain half-maximal uptake rate at a concentration of 30–40 mM Cl/liter. By contrast, the sodium uptake curve suggests a single mechanism, with a concentration necessary for half the maximal uptake rate of *ca.* 8–10 mM Na/liter, and becoming saturated at a concentration of *ca.* 40–50 mM Na/liter, at a rate of 6–7 μm Na/(g·hr). Curves illustrating such a two-mechanism hypothesis could easily be drawn, but the only real difficulty is that, except for the present curve (Figure 3), there is not a shred of evidence for it.

The remaining physiological hypothesis is that Cl-uptake below 10% SW might be depressed by or related to the opposing, inside-negative, body-wall potential

(Smith, 1970a; Fletcher, 1970). Were this potential linearly related to external salinity, no such localized depression in the exchange curve would be expected, but Fletcher's data show that the inside-negative potential is negligible down to the low external Cl-concentration of *ca.* 50–60 mM Cl/liter (10% SW), and then rises to nearly 50 mV inside-negative as external Cl-concentrations fall to 1 mM Cl/liter (*ca.* 0.2% SW). This range of rising potential corresponds exactly to the range in which the depression of the Cl-exchange curve is seen. Fletcher's data are thus compatible with the idea that the body wall potential has an important relationship to the uptake of chloride by *N. diversicolor* at very low salinities. Possibly the inside-negative potential is itself the result of the active inward transport of chloride, the uptake of sodium being incapable of producing such a potential.

Activation of the Na-uptake mechanism of *N. diversicolor* at low salinities has not been demonstrated, but this failure to show activation of Na-uptake does not prove its absence. The assumption that the method used might show an activation of uptake rested upon the prior assumption that other factors, such as permeability to the passage of Na, are not greatly altered. However, the latter assumption is not tenable because the permeability of the body wall to sodium is decreased at low salinities (Oglesby, 1970; this study). If, in adapting to 0.2% SW, *N. diversicolor* establishes a permeability to Na sufficiently lower than that in 3 or 5% SW, then the return to a higher salinity might find it unable to achieve the rate of inward transport expected at that higher salinity. The result, in terms of Na-influx, would be the resultant of any activation of the Na-uptake mechanism together with any reduction of Na-movement resulting from decreased permeability. The results of these experiments are compatible with the hypothesis that a sodium-permeability lowering in very low salinities (*e.g.,* in 0.2 and 1% SW), is sufficiently long-lasting to depress the uptake in 3 and 5% SW for at least the hour of the test in these experiments. The slight tendency for an elevation of uptake in the transfer from 0.2 to 1% SW might suggest an activation of uptake, with a small enough difference in sodium permeability to permit the activation to be revealed. Thus, while the overall result of a depressed Na-uptake after transfer from a very low to a higher salinity seems best explained by Na-permeability reduction as the principal adaptation to the very low salinity, the possibility of activation of the Na-uptake mechanism is not excluded. The decreased permeability characterizing adaptation to extremely low salinities limits the uptake to less than what the transport system could carry when more sodium is suddenly made available, and must tend to mask any activation of uptake. Experiments of this type cannot, without additional information, distinguish between absence of activation and the masking of adaptation by altered permeability.

In possible contrast to these results, Oglesby (1972) has reported an augmentation of Na-exchange after transfer of *N. diversicolor* from short exposures in deionized water back to adaptational salinities of 25% SW or higher. This phenomenon does not appear comparable to the present experimental results. However, when Oglesby exposed worms adapted in 0.5–6% SW to deionized water for 2–4 hr and then returned them to their adaptational media, he found that they resumed their characteristic efflux constant immediately. They thus seem to show considerable stability in respect to variation in the low-salinity range, and further study is needed to clarify the matter.

These studies were carried out at Zoophysiological Laboratory A, August Krogh Institute, University of Copenhagen, Denmark. I am indebted to Professor C. Barker Jørgensen and Lecturer Jørgen Gomme for much help, cooperation, and stimulating discussion. I thank Drs. Lars Ole Simonsen of Zoophysiological Laboratory B for permission to use flame photometer and chloride titrator. Partial support of the research by a NATO Senior Fellowship and by the Committee on Research, Berkeley Campus, of the University of California is gratefully acknowledged, as is also the housing provided by Danmarks Nationalbank.

SUMMARY

1. Experiments to compare the exchange (total influx) of sodium and chloride in the polychaete *Nereis diversicolor* in steady-state adaptation to very low salinities are reported.

2. The Na-uptake mechanism shows a high affinity for sodium, reaching half the maximal uptake rate at an external Na-concentration of 8–10 mM/liter (*ca.* 2% SW), and becomes "saturated" or reaches a plateau of uptake at concentrations of 40–50 mM/liter (*ca.* 10% SW) up to *ca.* 350 mM/liter (75% SW), above which Na-exchange is proportional to the external concentration.

3. The Cl-uptake curve differs from the Na-uptake curve in showing a relative depression at very low salinities before reaching "saturation" at Cl-concentrations of 50–60 mM/liter (*ca.* 10% SW). Cl-uptake becomes proportional to external concentration in salinities of 50% SW or greater, suggestive of passive diffusion in the ionic and osmotic conforming range.

4. It is shown that the permeability of the body wall, both to Na and to Cl, is reduced at very low salinities, thus destroying one of the assumptions upon which a previously-presented balance-sheet for chloride exchanges in *N. diversicolor* was based (Smith, 1970a).

5. Attempts to demonstrate an activation of the Na-uptake mechanism at very low salinities were inconclusive; reduction of body-wall permeability to sodium masks any possible activation.

6. It is suggested that the inside-negative body-wall potential is related to the depression of the Cl-uptake curve in salinities below 10% SW.

LITERATURE CITED

AHEARN, G. A. AND J. GOMME, 1975. Transport of exogenous D-glucose by the integument of a polychaete worm (*Nereis diversicolor* Müller). *J. Exp. Biol.*, **62**: 243–264.

FLETCHER, C. R., 1970. The regulation of calcium and magnesium in the brackish water polychaete *Nereis diversicolor* O. F. M. *J. Exp. Biol.*, **53**: 425–443.

FLETCHER, C. R., 1974a. Volume regulation in *Nereis diversicolor*—1. The steady state. *Comp. Biochem. Physiol.*, **47A**: 1199–1214.

FLETCHER, C. R., 1974b. Volume regulation in *Nereis diversicolor*—2. The effect of calcium. *Comp. Biochem. Physiol.*, **47A**: 1215–1220.

FLETCHER, C. R., 1974c. Volume regulation in *Nereis diversicolor*—3. Adaptation to a reduced salinity. *Comp. Biochem. Physiol.*, **47A**: 1221–1234.

HALE, L. J., 1958. *Biological laboratory data.* Wiley, New York, 132 pp.

JØRGENSEN, C. B. AND R. P. DALES, 1957. The regulation of volume and osmotic regulation in some nereid polychaetes. *Physiol. Comp. Occol.,* **4**: 357–374.

OGLESBY, L. C., 1969a. Inorganic components and metabolism; ionic and omsotic regulation: Annelida, Sipuncula, and Echiura. Pages 211–310 in M. Florkin and B. T. Scheer, Eds., *Chemical zoology, Vol. 4.* Academic Press, New York.

OGLESBY, L. C., 1969b. Salinity-stress and desiccation in intertidal worms. *Amer. Zool.,* **9**: 319–331.

OGLESBY, L. C., 1970. Studies on the salt and water balance of *Nereis diversicolor*—I. Steady-state parameters. *Comp. Biochem. Physiol.,* **36**: 449–466.

OGLESBY, L. C., 1972. Studies on the salt and water balance of *Nereis diversicolor*—II. Components of total sodium efflux. *Comp. Biochem. Physiol.,* **41A**: 765–790.

POTTS, W. T. W AND G. PARRY, 1964. *Osmotic and ionic regulation in animals.* Pergamon Press, London, 423 pp.

RASMUSSEN, E., 1973. Sytematics and ecology of the Isefjord marine fauna (Denmark). *Ophelia,* 11: xvi + 1–495.

SHAW, J. AND D. W. SUTCLIFFE, 1961. Studies on sodium balance in *Gammarus duebeni* (Lilljeborg) and *G. pulex* (L.). *J. Exp. Biol.,* **38**: 1–15.

SMITH, R. I., 1955a. On the distribution of *Nereis diversicolor* in relation to salinity in the vicinity of Tvärminne, Finland, and the Isefjord, Denmark. *Biol. Bull.,* **108**: 326–345.

SMITH, R. I., 1955b. Comparison of the level of chloride regulation by *Nereis diversicolor* in different parts of its geographical range. *Biol. Bull.,* **109**: 453–474.

SMITH, R. I., 1970a. Chloride regulation at low salinities by *Nereis diversicolor* (Annelida, Polychaeta). I. Uptake and exchanges of chloride. *J. Exp. Biol.,* **53**: 75–92.

SMITH, R. I., 1970b. Chloride regulation at low salinities by *Nereis diversicolor* (Annelida, Polychaeta). II. Water fluxes and apparent permeability to water. *J. Exp. Biol.,* **53**: 93–100.

SMITH, R. I., 1970c. Hypo-osmotic urine in *Nereis diversicolor.* *J. Exp. Biol.,* **53**: 101–108.

Reference: *Biol. Bull.* **151**: 601–614. (December, 1976)

OSMOTIC ADJUSTMENT IN AN ESTUARINE POPULATION OF *UROSALPINX CINEREA* (SAY, 1822) (MURICIDAE, GASTROPODA)

KENNETH W. TURGEON[1]
Jackson Estuarine Laboratory, University of New Hampshire, Durham, New Hampshire 03824

Marine and estuarine molluscs have long been considered to be strict osmotic conformers with a limited degree of ionic regulation (Prosser and Brown, 1961; Potts and Parry, 1964). However, studies by Todd (1964) and Peterson and Duerr (1969) suggest that this generalization may have to be modified in light of their experimental findings. Unfortunately, neither study yielded definitive results, and the question of possible osmotic regulation for some species of marine and estuarine molluscs is still unanswered.

Todd (1964) demonstrated that marsh periwinkles, *Littorina littorea*, held in sea water of 8.8‰ salinity had blood osmolalities as much as three times greater than that of the external medium. These results as indicators of osmotic regulation are debatable, since *L. littorea* is capable of completely sealing itself off from the environment by withdrawing into the shell and plugging the shell aperture with its operculum. Thus, it is conceivable that the test animals were not responding physiologically to the salinity but had simply "isolated" themselves from it through a morphological-behavioral adaptation. This shutting off from the environment is typical of many intertidal molluscs and serves to prevent desiccation when the animals are exposed to the atmosphere during periods of low tide.

Peterson and Duerr (1969) suggested osmotic regulation in *Tegula funebralis*, an intertidal limpet, on the basis of a decrease in free amino acid content of the tissue when the test animals were transferred from water having a salinity 120% that of normal sea water to 160% strength. Their contention of osmotic regulation was based on the known role of free amino acids and other small organic ions as osmotic effectors of intracellular fluids of marine and estuarine invertebrates (Duchâteau, Sarlet, Camien and Florkin, 1952; Lewis, 1952; Duchâteau and Florkin, 1955, 1956; Potts, 1958; Shaw, 1958; Simpson, Allen and Awapara, 1959; Allen, 1961; Awapara, 1962; Lange, 1963; Lynch and Wood, 1966; Schoffeniels, 1967). However, they did not investigate salinity-induced changes of those inorganic ions which are major osmotic effectors of both intracellular and extracellular body fluids (Potts and Parry, 1964; Robertson, 1964). Also, the decrease in free amino acid content was not accompanied by a decrease in total ninhydrin-positive substances indicating that other, unidentified organic ions varied directly with salinity. The observed inverse relationship between salinity and free amino acid content does not, by itself, lend much support to their contention of osmotic regulation in *T. funebralis*. They stated that further studies of this nature should investigate salinity-induced changes in those organic and inorganic ions which are major osmotic effectors of tissue fluids. Such a study was conducted by Gilles

---

[1] Present appointment: Department of Biology, American University of Beirut, Beirut, Lebanon. Present mailing address (for reprints, etc.): 2959 Pinellas Point Drive S., St. Petersburg, Florida 33712.

(1972) on three species of pelecypod molluscs, and he found no evidence of osmotic regulation for any of the three species studied. Yet, it does not seem feasible to look to pelecypods for osmotic regulation since most species have the ability to either shut themselves off from the environment or burrow into the substratum if the environment becomes stressful. This author has observed the ability of oysters to remain in air at 10° C for two weeks and then be returned to their normal aquatic environment, to survive and grow. Robertson (1964) suggested that if osmotic regulation does occur within some marine and estuarine mollusc species, it would more likely be found among the estuarine prosobranch gastropods. Osmotic regulation within the true marine forms is not expected since evolutionary adaptation to the stenohaline conditions of the marine environment would favor osmotic conformity rather than osmotic regulation.

The present study was conducted in response to the suggestions of Robertson (1964) and Peterson and Duerr (1969). It investigated salinity-induced changes in major organic and inorganic osmotic effectors of tissue fluids of an estuarine prosobranch gastropod species. The species chosen for study was the common oyster drill, *Urosalpinx cinerea* (Say, 1822), a highly active, predatory animal incapable of closing itself off completely from the environment due to the presence of a siphonal canal. The population from which the test animals were taken occurs subtidally in Great Bay, New Hampshire. The salinity regime of the population ranges from about 5‰ during spring freshets to 30‰ in late summer. Periods of reduced salinities of less than 10‰ during spring may approach four weeks duration (personal observation) depending on accumulated snowfall, ice cover, rate of melting and spring rains.

MATERIALS AND METHODS

Animals were collected from the field four weeks prior to the start of the study and acclimated in the laboratory to a salinity of 30 ± 0.1‰ and a temperature of 20 ± 1° C. Ambient salinity at the time of collection was 28.6‰ and temperature was 25° C. Test animals were chosen for similarity of size and ranged from 22 to 29 mm in shell length. During the acclimation period and the study, the animals were held in 45 liter acryllic plastic aquaria, maintained at a density of three individuals or less per liter of water and fed small (15–25 mm in shell height) *Mytilus edulis.* Nylon screening, secured just below the surface of the water, prevented the animals from crawling upwards out of the water column. Aerated, standing water was used, and the desired salinities were prepared five hours before use by either dilution of Great Bay water with well water or addition of artificial sea salt (Aquarium Systems, Inc.) to Great Bay water. Atomic absorption analysis of the experimental salinities and ambient Great Bay water showed that the relative proportions of the major inorganic ions did not differ significantly (less than 5% variation) from that of "normal" sea water (see Barnes, 1954). Salinities were checked for accuracy (± 0.1‰) by titration with silver nitrate and adjusted if necessary before use. Osmolalities of the test salinities were determined in a semi-automatic osmometer (Advanced Instruments, Inc., Model 3-W). Half of the water in the aquarium was changed every four days during the extended acclimation period. Aquaria were covered with acryllic plastic tops to prevent evaporative loss of water. The pH of all laboratory prepared water never fell below 7.1 nor exceeded 7.9; these limits are well within the survival range for *U. cinerea* (Sizer, 1936).

The test animals were divided into two groups at the end of the four week acclimation period. One group was subjected to 2.5‰ sequential increases in salinity up to a final salinity of 40‰. The other group was subjected to 2.5‰ sequential decreases in salinity down to a final salinity of 10‰. Ten animals per salinity were randomly sampled for osmotic adjustment at 120 hours exposure to the test salinities of 40, 35, 25, 20, 15 and 10‰, and the tissues were analyzed to determine osmotic adjustment. The remaining animals were then transferred to the next salinity in the series. Exposure time to each of the inbetween salinities (37.5, 32.5, 27.5, 22.5, 17.5 and 12.5‰) was 72 hours. Ten animals were removed from the acclimation salinity of 30‰ just prior to the start of the study, and the tissues were analyzed for initial "osmotic condition."

Osmotic adjustment was determined by recording changes in tissue fluid osmolality, percentage water content and tissue levels of chloride, sodium, potassium, ninhydrin-positive substances (NPS) and free amino acids (FAA). All of these analyses, with the exception of FAA, were conducted on individual animals. FAA determinations were carried out on pooled homogenates of the five tissues. The other five tissues were individually analyzed for percentage water content. The mean water content of the tissues was used to convert tissue constituent values for the other five animals from millimoles per kilogram wet tissue to millimoles per kilogram tissue water (molality).

Preparation of tissue homogenates

Animals were cracked out of their shells, the opercula carefully removed and the tissues quickly rinsed in distilled water, blotted on absorbent paper and dried for two minutes under a stream of air at room temperature. During shell removal care was taken to sever only the muscles connecting the body to the columella to prevent excessive tissue damage and bleeding. The wet tissue was weighed to the nearest mg and then homogenized in a volume of doubly distilled water ten times the wet weight of the tissue. Thus, all tissues were identically diluted 11-fold with distilled water on a weight-to-weight basis. Homogenization was by a motor-driven pestle, and the homogenizing tube was suspended in an icewater bath. The homogenates were placed in air-tight vials and stored in a freezer at –20° C until the actual tissue analyses were performed. Just prior to analysis for tissue constituents the homogenates were removed from the freezer, quickly thawed in warm water and centrifuged in a Sorvall high speed, refrigerated centrifuge (Model RC2–B) at 10,000 rpm (RCF = 12,100) for 30 minutes at 4° C. The pellet was discarded, and fractions of the supernatant were taken for each of the specific analyses mentioned earlier.

Prior to each use all glassware used in the analyses was soaked for several hours in a sulfuric acid-potassium dichromate cleaning solution, soaked and rinsed in a doubly distilled water and then inverted and allowed to air dry.

Tissue water content

Animals were removed from their shells and treated as described in the homogenization section with the exception that they were not homogenized or frozen. The whole tissues were dried in an oven at 90° C for 72 hours, allowed to cool in a desiccator and then reweighed as dry tissues. Water content was calculated as the difference between wet tissue weight and dry tissue weight and expressed as a percentage of the wet tissue weight.

Tissue fluid osmolality

Osmolality of the supernatant was determined in a semi-automatic osmometer (Advanced Instruments, Inc., Model 3-W). Only 0.25 ml of the supernatant was required. Conversion of supernatant osmolality to tissue fluid osmolality was calculated according to the following formula: theoretical dilution factor of tissue water based on mean tissue water content of the other five animals × the osmolality of the homogenate in mOsmoles = tissue osmolality in mOsmoles. The resulting osmolalities were higher than the true tissue fluid osmolalities because of the inclusion of solutes normally not osmotically active (*e.g.,* calcium carbonate granules stored in certain cells of the digestive tract). Also, the ionic coefficient of an electrolyte solution increases as the solution becomes more dilute (Prosser and Brown, 1961).

Chloride, sodium and potassium

A 0.2 ml fraction of the supernatant was diluted to two ml with doubly distilled water and tested for chloride by the titration method of Schales and Schales (1941). Sodium and potassium were determined in a Coleman flame photometer (Model 21) with a Coleman Galv-o-meter attachment (Model 22) according to the procedures developed by Coleman Instruments, Inc., and listed in the instruction manual accompanying the instrument. For sodium a 0.2 ml fraction of the supernatant was diluted to five ml with a 0.02% Flaminox solution (a nonionic detergent), for potassium a 0.2 ml fraction of the supernatant was diluted to ten ml with a 0.02% Flaminox solution.

These methods, while allowing for simplicity and rapidity of analysis, were not suitable for the determination of total chloride, sodium and potassium levels. Some unknown percentage of each element was most probably complexed with tissue proteins or other organics which were spun-down during centrifugation of the distilled water diluted homogenates. However, these three elements occur mostly as free, noncomplexed ions in extracellular and intracellular body fluids (Steinbach, 1962; Oser, 1965). Losses due to centrifugation were considered to be inconsequential to the scope of this study since complexed forms of chloride, sodium and potassium would be osmotically inactive anyway. Deproteinization of the supernatant fractions taken for the chloride, sodium and potassium determinations was obviated by the final dilution of these samples prior to photometric analysis (see Schales and Schales, 1941; Hermann and Alkemade, 1963).

Ninhydrin-positive substances (NPS)

A 0.2 ml fraction of the supernatant was brought up to four ml with 75% ethyl alcohol, mixed well and heated for ten minutes at 80° C to precipitate protein. The homogenate-alcohol mixture was centrifuged for eight minutes at

5000 rpm (RCF = 3020), and the supernatant was decanted and saved. The remaining pellet was washed with two ml of 75% ethyl alcohol, recentrifuged and the washing added to the first supernatant obtained. The combined supernatant (original plus washing) was evaporated to just dryness in a drying oven at 80° C. The residue present in the bottom of the tube was redissolved in eight ml of doubly distilled water and tested for NPS by the method of Troll and Cannan (1953). Absorbance was measured in a Bausch and Lomb Spectronic 20 colorimeter at a wavelength of 570 millimicrons. Several concentrations of L-leucine were used to prepare a standard curve.

Free amino acids (FAA)

A 0.2 ml fraction was taken from each of the five supernatants, and these fractions were combined to yield a one ml pooled sample. A 0.2 ml fraction was then taken from this pooled sample and treated as outlined in the procedure for NPS. The dried residue was dissolved in four ml of a 2.2 pH citrate buffer and analyzed for individual free amino acids in a Beckman automatic amino acid analyzer (Model 120C) using the standard, protein hydrolyzate run (Spackman, Stein and Moore, 1958). Taurine, a sulfonic acid, is included in the FAA category.

RESULTS

Only four animals died during the course of this study. One death per salinity was recorded at the salinities of 40, 35, 22.5 and 15‰. Behavior was "normal" within the salinity range of 20 to 35‰. Animals transferred to the 15 and 40‰ salinities exhibited reduced feeding and crawling activity, but 75 to 80% of the animals were attached to the substratum at the end of 120 hours exposure. The

TABLE I

Mean tissue concentrations and ranges of the individual "osmotic" variables in test animals from the Great Bay, New Hampshire population of Urosalpinx cinerea *for each of the experimental salinities.*

Tissue variable	Salinity (‰) and Osmolality (mOsmole)						
	10‰ 289 mOsmole	15 435	20 579	25 726	30 879	35 1025	40 1180
Osmolality (mOsmole)	628	787	979	1159	1210	1389	1419
	613–670	761–806	931–1016	1028–1226	1175–1309	1329–1468	1309–1539
Water content (%)	77.5	76.6	74.9	73.1	71.9	70.8	70.3
	75.5–80.0	70.5–81.1	71.6–76.7	70.0–76.2	67.8–76.7	66.7–75.5	69.1–72.2
Chloride*	105.7	112.7	152.1	212.6	234.9	291.8	309.0
	95.6–113.4	96.9–126.8	138.8–159.9	195.8–234.3	209.2–263.5	260.4–311.3	272.9–351.4
Sodium*	97.9	119.8	152.1	209.8	218.1	222.1	244.8
	81.4–109.6	115.2–127.9	126.5–173.0	194.1–240.6	192.7–244.8	204.0–243.4	226.9–263.0
Potassium*	69.5	73.3	78.8	91.0	93.6	99.8	100.9
	66.4–71.8	70.0–74.5	71.8–90.0	85.4–94.7	88.2–105.0	94.5–103.9	97.6–107.7
Total FAA*	28.9	42.6	47.9	103.2	127.0	124.1	138.8
NPS*	54.1	62.1	55.4	123.3	130.6	151.3	163.9
	41.6–66.5	56.3–73.3	48.6–68.6	118.5–129.0	107.2–170.9	130.3–167.5	147.8–184.8
Total FAA as a percentage of NPS	53.5	68.7	86.4	83.7	97.3	82.0	84.7

* mM/kg tissue water.

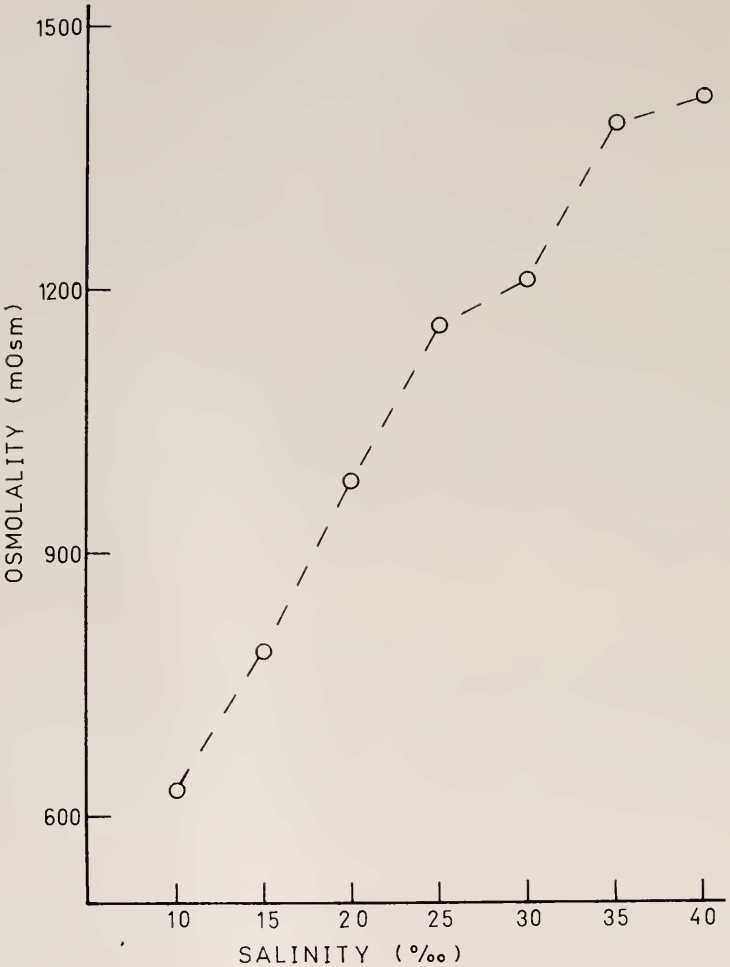


FIGURE 1. Salinity-induced changes in the mean tissue fluid osmolality of test animals from the Great Bay, New Hampshire population of *Urosalpinx cinerea.*

animals transferred to the 10‰ salinity displayed an immediate stress response and exhibited no feeding activity. However, by the end of 120 hours exposure at 10‰ approximately 40% of the animals were attached to the substratum and all exhibited a complete withdrawal into the shell when subjected to a tactile stimulus.

Changes in mean tissue fluid osmolality and mean tissue concentrations of chloride, sodium and potassium followed the direction of the salinity changes (Table I; Figures 1 and 2). These variables increased with increasing salinity and decreased with decreasing salinity. Changes in mean tissue water content approximated an inverse, linear relationship with salinity (Table I; Fig. 3).

Changes in tissue concentrations of FAA followed the direction of the salinity changes with one exception; the FAA concentration at the 35‰ salinity was

2.9 mM/kg tissue water less than the FAA concentration at the 30‰ salinity (Table I; Fig. 4). Changes in mean tissue concentrations of NPS followed the direction of the salinity changes between 25 and 40‰, but the mean NPS concentration at 20‰ was 6.7 mM/kg tissue water less than that at 15‰ (Table I; Fig. 4). The mean NPS concentration at 10‰ was only 1.3 mM/kg tissue water less than the 20‰ concentration.

Differences in magnitude between the 40‰ and 10‰ mean tissue concentrations of the variables investigated are as follows: tissue fluid osmolality 2.3; chloride, 2.9; sodium, 2.5; potassium, 1.4; FAA, 4.8; NPS, 3.0 and 1.1 for percentage water content. It is apparent from this list that FAA exhibited the greatest relative change within the salinity range of this study, while the percentage of water content exhibited the least relative change. Of the inorganic ions investigated, potassium showed the least change with salinity and chloride the most change. The total difference between the 40‰ and 10‰ mean tissue concentrations

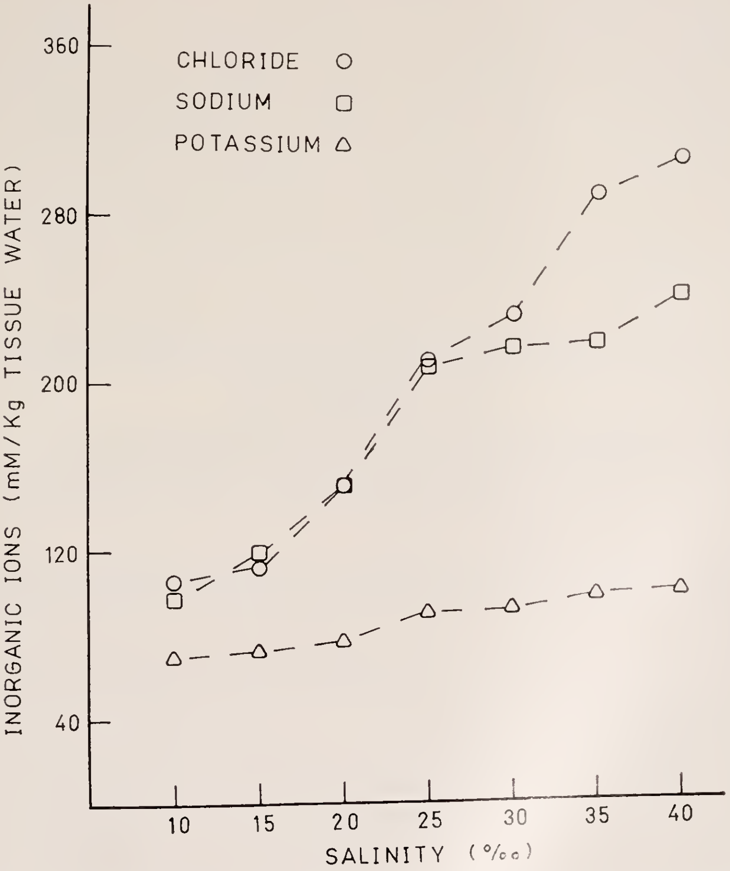


FIGURE 2. Salinity-induced changes in the mean tissue concentrations of chloride, sodium and potassium of test animals from the Great Bay, New Hampshire population of *Urosalpinx cinerea*.

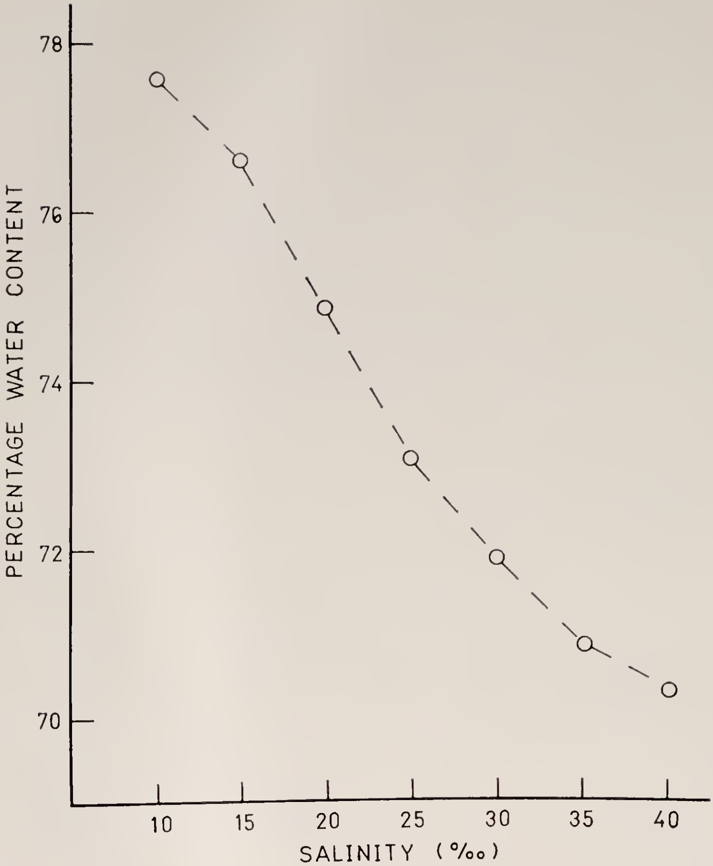


FIGURE 3. Salinity-induced changes in the mean tissue water content of test animals from the Great Bay, New Hampshire population of *Urosalpinx cinerea.*

of potassium was 31.4 mM/kg tissue water, whereas these differences for chloride and sodium were 203.3 and 146.9 mM/kg tissue water, respectively. Mean tissue chloride concentrations at the salinities of 25, 20, 15 and 10‰ were less than 8 mM/kg tissue water different from the corresponding sodium concentrations. But, the chloride concentrations at the salinities of 30, 35 and 40‰ were, respectively, 16.8, 69.7 and 64.2 mM/kg tissue water greater than the corresponding sodium concentrations.

FAA contributions to the mean tissue concentrations of NPS varied from 53.5 to 97.3%. The largest percentage contribution occurred at the acclimation salinity of 30‰. The lowest contributions occurred at the 15‰ (68.7%) and 10‰ (53.5%) salinities. Percentage contributions for the other four salinities were similar and ranged between 82.0 and 86.4%. Taurine was the most abundant amino acid at all experimental salinities (Table II) and comprised from 36.6 to 52.7% of the FAA pools. Other amino acids present in relatively consistent high

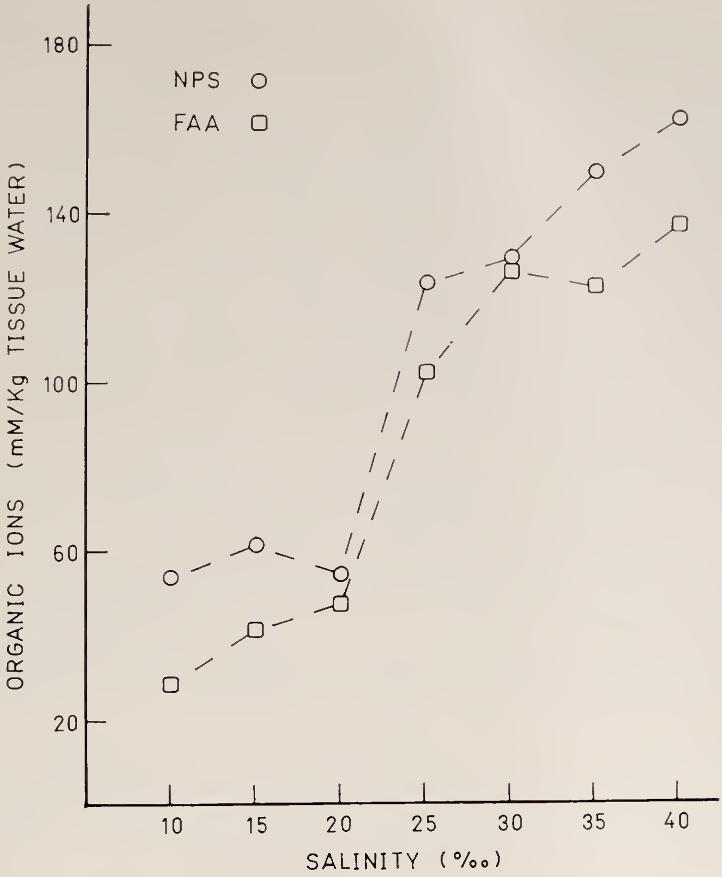


FIGURE 4. Salinity-induced changes in the mean tissue concentrations of total free amino acids (FAA), including the sulfonic acid taurine, and ninhydrin-positive substances (NPS) of test animals from the Great Bay, New Hampshire population of *Urosalpinx cinerea.*

amounts were aspartic acid, alanine and glycine. These three amino acids plus taurine comprised from 59.5 to 75.7% of the total FAA pools.

DISCUSSION

Because measurements of all tissue variables were conducted on whole tissue homogenates, it is impossible to discern the intracellular and extracellular distribution of the individual ionic "species". However, it is known that inorganic ions, primarily chloride and sodium, are the major osmotic effectors of the blood of marine and estuarine invertebrates (including molluscs), while FAA and other small organic ions are major osmotic effectors of the intracellular fluids (Potts and Parry, 1964; Robertson, 1964; Florkin, 1966; Schoffeniels, 1967). Thus, the intracellular and extracellular fluids are isosmotic with each other but differ in the types and concentrations of ions responsible for the respective osmolalities.

Salinity-induced variations in tissue concentrations of all osmotic ions investigated and tissue fluid osmolality were greater than those expected due to changes in tissue water content alone. Osmotic adjustment to the various test salinities must have included not only changes in tissue water content but also external-internal fluxes of the ions themselves. Ionic exchanges between the external medium and internal body fluids would "buffer" the degree of tissue hydration or dehydration and allow the cells to become isotonic with the external medium after an initial period of osmotic and ionic adjustment. In this regard severe tissue hydration or dehydration did not appear to be a problem confronting the test animals, and changes in tissue water content were much less than would be expected if the animals had acted as perfect osmometers with no mechanism of volume regulation. Test animals at the low salinities of 15 and 10‰ did not appear swollen and, upon tactile stimulation, could retract body and foot completely into the shell. At this point it is worth mentioning that no deaths occurred at the minimum test salinity of 10‰ during this study, and that the 11 "extra" animals remaining at the 10‰ salinity were held at this salinity for an additional two weeks before the first death was recorded. Surprisingly, one (or perhaps two) of these animals deposited two egg cases during this additional holding period at the 10‰ salinity. The viability of these egg cases was not investigated.

The nearly linear relationship between tissue chloride concentration and salinity and the large order of magnitude of change in tissue chloride over the range of test salinities shows that chloride ions were freely exchangeable between the external medium and the internal milieu. Robertson (1949) investigated ionic regulation in six species of marine molluscs, including two species of prosobranch

TABLE II

Tissue concentrations of individual free amino acids in test animals from the Great Bay, New Hampshire population of Urosalpinx cinerea *for each of the experimental salinities. Concentrations are in* mM*/kg tissue water; tr indicates traces; dashed lines, not detected.*

Amino acid	Salinity (‰)						
	10	15	20	25	30	35	40
Alanine	1.77	2.74	2.74	9.17	10.56	9.17	15.28
Arginine	0.63	0.79	tr	0.35	1.49	0.26	0.92
Aspartic acid	4.01	4.42	7.40	9.31	11.45	9.56	10.04
Cysteic acid	1.61	2.16	6.28	tr	2.51	3.05	2.30
Glutamic acid	1.87	2.69	3.62	5.23	5.09	3.75	3.87
Glycine	1.52	2.51	0.90	8.62	14.56	11.58	14.38
Histidine	—	—	tr	0.23	0.39	tr	0.64
Isoleucine	0.49	0.18	0.36	1.56	1.62	1.68	1.98
Leucine	0.62	0.46	0.44	2.34	2.37	2.24	2.62
Lysine	1.19	1.25	0.50	5.35	4.31	3.78	3.44
Phenylalanine	—	—	tr	0.43	0.45	0.39	0.32
Proline	—	tr	tr	4.15	3.53	3.06	7.04
Serine	0.45	1.32	1.81	4.97	4.82	4.47	6.17
Threonine	0.26	0.85	1.04	2.58	3.63	3.85	4.31
Tyrosine	—	—	tr	0.39	0.35	0.37	0.34
Valine	—	0.42	4.20	2.40	2.68	2.74	3.40
Taurine	14.49	22.47	17.52	45.96	57.07	63.71	61.27

gastropods, and found that they did not regulate blood chloride levels. His data showed that the blood chloride concentrations of these species were within ±1% that of the external medium. The order of magnitude change in tissue chloride concentration in the present study was 2.9 between the extreme salinities of 10 and 40‰. This represents a rather large change, especially since the measurements were made on whole tissue homogenates rather than on just blood samples. Thus, it is concluded that the experimental animals did not regulate blood levels of chloride but that the blood chloride concentrations were, for all practical purposes, identical to that of the external medium over the range of test salinities employed.

Tissue sodium showed the same relationship with salinity as did chloride between the salinities of 10 and 25‰ but between 25 and 30‰ the slope of the linear relationship decreased sharply and remained decreased up through the 40‰ salinity. These data suggest a nonregulation of blood sodium levels between 10 and 25‰ and a hyporegulation between 25 and 40‰. Robertson (1949) found a slight (3%) hyporegulation of blood sodium in *Buccinum undatum*, another marine prosobranch gastropod, at a salinity of approximately 32‰ but did not test for any possible variation in blood sodium regulation at other salinities. If, in fact, the experimental specimens of *U. cinerea* did exhibit hypo-ionic regulation of blood sodium at the test salinities of 25 to 40‰, then there must have occurred either an increased hyper-ionic regulation of some other cation(s) or an increased hypo-ionic regulation of some anion(s) or both if the electro-chemical balance of the blood was to be maintained.

Although tissue potassium varied linearly with salinity, the order of magnitude change between consecutive experimental salinities was small. Much of the change in tissue potassium concentration with salinity can be accounted for by change in tissue water content. Potts (1958) found that most of the salinity-induced change in potassium concentration of *Mytilus edulis* adductor muscle could be accounted for by change in tissue water content. Marine molluscs studied to date concentrate potassium at levels several times greater than that of the surrounding sea water (Potts and Parry, 1964). The internal-external distribution of potassium is intracellular > extracellular > sea water. In the present study tissue potassium concentrations were several times greater than those of the respective experimental salinities. Data presented by Hayes and Pelluet (1947) showed that the potassium concentration of *B. undatum* foot muscle was approximately 11-times greater than that of the blood. Robertson (1949) found that *B. undatum* exhibited a large (42%) hyper-ionic regulation of blood potassium compared to the external medium. In light of the above information, it is suggested that the experimental specimens of *U. cinerea* exhibited a substantial degree of hyper-ionic regulation of tissue potassium, and that the exchange of potassium ions between the internal milieu and external medium was quite limited over the entire range of test salinities. Such a mechanism of nearly steady-state hyper-regulation would greatly limit the osmotic function of potassium to changes in external salinity. Gilles (1972) observed the blood and perivisceral potassium levels of two species of marine bivalves to remain nearly constant over a salinity regime of 100 to 25% sea water and has also questioned the osmoregulatory importance of potassium regulation in marine molluscs.

FAA concentration showed a continuous decrease with salinity between the 30 and 10‰ salinities, whereas NPS concentration leveled off between 20 and 10‰.

This resulted in a substantial decrease in FAA contribution to the NPS pools at the 15 and 10‰ salinities and suggests that other, unidentified nitrogenous compounds were major contributors to intracellular osmolality at these low salinities. The compounds involved may be the same as or similar to the dialyzable, unidentified compounds found in *Mytilus edulis* (Briceteux-Grégoire, Duchâteau, Jeuniaux and Florkin, 1964) and *Tegula funebralis* (Peterson and Duerr, 1969). It is worth noting that the highest FAA contribution occurred at the acclimation salinity of 30‰. It is unknown if other FAA contributions would have approached this maximal level if the period of exposure to the other salinities had been extended.

The relative abundances of the individual FAA obtained in this study are in partial agreement with those for other marine prosobranch gastropods. Simpson, Allen and Awapara (1959) found taurine, alanine, arginine, aspartic acid, glutamic acid and glycine to be the six most abundant FAA in *Thais haemastoma, Polinices duplicata* and *Oliva sayana.* Peterson and Duerr (1969) found a similar FAA distribution in *Tegula funebralis.* The major difference between the FAA results of the present study and these other studies is the relative concentrations of arginine. In the present study arginine concentrations were consistently low, whereas these other investigators found arginine to be a major component of the FAA pools of the above four species. The low arginine values are unexpected, since one would suppose arginine phosphate to be present in the muscles. Several, preliminary "long column" runs in the amino acid analyzer revealed the presence of ornithine, a known metabolite of arginine hydrolysis (Tschiersch and Mothes, 1963), at levels up to 5 mM/kg tissue water in animals held at the acclimation salinity of 30‰. Thus, it is conceivable that much of the arginine was converted, through hydrolysis, to ornithine and other metabolites. This would account for the low levels of arginine obtained.

The large salinity-induced changes in FAA concentrations observed in this study show that these organic compounds were major components of the intracellular osmolality of the test animals. Robertson (1964; based on data of Potts, 1958) calculated that the FAA pool comprised 47.6% of the major osmotic constituents in *Mytilus edulis* fast adductor muscle in full-strength sea water and 54.0% in half-strength sea water.

The tissue fluid osmolality, chloride, sodium, potassium and FAA data of this study demonstrate that the laboratory population of *U. cinerea* did not exhibit anisosmotic regulation within the range of test salinities employed. The slight decrease in total FAA concentration between the 30 and 35‰ salinities does not warrant a contention of attempted intracellular anisosmotic regulation, since FAA constitute only a portion of the total intracellular osmotic effector pool. It is postulated that the Great Bay population of *U. cinerea* does not maintain its euryhaline survival status through an osmoregulatory mechanism. Rather, the population has probably adapted physiologically to withstand dilution of its body fluids during spring conditions of low salinities.

SUMMARY

Individuals from a subtidal, estuarine population of the common oyster drill, *Urosalpinx cinerea* (Say, 1822), were brought into the laboratory and tested for

osmotic adjustment to changing salinity. Tissue variables monitored at seven experimental salinities ranging from 10 to 40‰ were tissue fluid osmolality, chloride, sodium, potassium, free amino acids (FAA), ninhydrin-positive substances (NPS) and water content. The results of this study demonstrate that the test animals did not exhibit anisosmotic regulation at any of the experimental salinities. However, the data do suggest a high degree of hyper-ionic regulation of potassium at all experimental salinities and a hyporegulation of sodium between the 25 and 40‰ salinities. Taurine, aspartic acid, alanine and glycine were the four FAA present in relatively consistent high amounts. These four amino acids comprised from 59.6 to 75.7% of the total FAA pools.

It is postulated that the population does not maintain its euryhaline survival status through an osmoregulatory mechanism. Rather, the population has probably adapted physiologically to withstand dilution of its body fluids during spring conditions of low salinities.

LITERATURE CITED

Allen, K., 1961. The effect of salinity on the amino acid concentration in *Rangia cuneata* (Pelecypoda). *Biol. Bull.*, **121**: 419–424.

Awapara, J., 1962. Free amino acids in invertebrates: A comparative study of their distribution and metabolism. Pages 158–175 in J. T. Holden, Ed., *Amino acid pools.* Elsevier Publishing Company, Amsterdam, London, New York.

Barnes, H., 1954. Some tables for the ionic composition of sea water. *J. Exp. Biol.*, **31**: 582–588.

Bricteux-Grégoire, S., G. Duchâteau, C. Jeuniaux and M. Florkin, 1964. Constituants osmotiquement actifs des muscles adducteurs de *Mytilus edulis* adapté à l'eau de mer ou à l'eau saumâtre. *Arch. Intern. Physiol. Biochem.*, **72**: 116–123.

Duchâteau, G. and M. Florkin, 1955. Concentration du milieu extérieur et état stationnaire du pool des acides aminés non protéiques des muscles d'*Eriocheir sinensis,* Milne Edwards. *Arch. Intern. Physiol. Biochem.*, **63**: 249–251.

Duchâteau, G. and M. Florkin, 1956. Systèmes intracellulaires d'acides aminés libres et osmorégulation des crustacés. *J. Physiol.*, **48**: 520.

Duchâteau, G., H. Sarlet, M. N. Camien and M. Florkin, 1952. Acides aminés non protéiques des tissus chez les mollusques lamellibranches et chez les vers. Comparison des formes marines et des formes dulcicoles. *Arch. Intern. Physiol.*, **60**: 124–125.

Florkin, M., 1966. Nitrogen metabolism. Pages 309–343 in K. Wilbur and C. M. Yonge, Eds., *Physiology of Mollusca, Vol. II.* Academic Press, New York.

Gilles, R., 1972. Osmoregulation in three molluscs: *Acanthochitona descrepans* (Brown), *Glycymeris glycymeris* (L.) and *Mytilus edulis* (L.) *Biol. Bull.*, **142**: 25–35.

Hayes, F. R. and D. Pelluet, 1947. The inorganic constitution of molluscan blood and muscle. *J. Mar. Biol. Ass. U. K.*, **26**: 580-589.

Hermann, R. and C. T. J. Alkemade, 1963. *Chemical analyses by flame photometry.* Inter-science Publishers, New York, London, 644 pp.

Lange, R., 1963. The osmotic function of amino acids and taurine in the mussel, *Mytilus edulis. Comp. Biochem. Physiol.*, **10**: 173–179.

Lewis, P. R., 1952. The free amino acids of invertebrate nerve. *Biochem. J.*, **52**: 330–338.

Lynch, M., and L. Wood, 1966. Effects of environmental salinity on free amino acids of *Crassostrea virginica* Gmelin. *Comp. Biochem. Physiol.*, **19**: 783-790.

Oser, B. L., 1965. *Hawk's physiological chemistry.* McGraw-Hill Book Company, New York, Toronto, Sydney, London, 1472 pp.

Peterson, M. B. and F. G. Duerr, 1969. Studies on osmotic adjustment in *Tegula funebralis* (Adams 1854). *Comp. Biochem. Physiol.*, **28**: 633–644.

Potts, W. T. W., 1958. The inorganic and amino acid composition of some lamellibranch muscles. *J. Exp. Biol.*, **35**: 749–764.

Potts, W. T. W. and G. Parry, 1964. *Osmotic and ionic regulation in animals.* Pergamon Press, Oxford, 438 pp.

Prosser, C. L. and F. A. Brown, 1961. *Comparative animal physiology.* W. B. Saunders Co., London, Philadelphia, 688 pp.

Robertson, J. D., 1949. Ionic regulation in some marine invertebrates. *J. Exp. Biol.,* **26**: 182–200.

Robertson, J. D., 1964. Osmotic and ionic regulation. Pages 283–311 in K. Wilbur and C. M. Yonge, Eds., *Physiology of Mollusca, Vol. I.* Academic Press, London, New York.

Schales, O. and S. Schales, 1941. A simple and accurate method for the determination of chloride in biological fluids. *J. Biol. Chem.,* **140**: 879–884.

Schoffeniels, E., 1967. Cellular aspects of active transport: V. Some aspects of the regulation of the intracellular pool of free amino acids. Pages 168–178 in M. Florkin and H. S. Mason, Eds., *Comparative biochemistry, Vol. VII.* Academic Press, London, New York.

Shaw, J., 1958. Osmoregulation in the muscle fibers of *Carcinus maenas. J. Exp. Biol.,* **35**: 920–929.

Simpson, J. W., K. Allen and J. Awapara, 1959. Free amino acids in some aquatic invertebrates. *Biol. Bull.,* **117**: 371–381.

Sizer, I. W., 1936. Observations on the oyster drill with special reference to its movement and to the permeability of its egg case membrane. Unpub. Report, U. S. Bur. Fish., Wash., D. C.

Spackman, D. H., W. H. Stein and S. Moore, 1958. Automatic recording apparatus for use in the chromatography of amino acids. *Anal. Chem.,* **30**: 1190.

Steinbach, H. B., 1962. Comparative biochemistry of the alkali metals. Pages 677–720 in M. Florkin and H. S. Mason, Eds., *Comparative biochemistry, Vol. IV.* Academic Press, London, New York.

Todd, M. E., 1964. Osmotic balance in *Lottorina littorea, L. littoralis* and *L. saxatilis* (Littorinidae). *Physiol. Zool.,* **37**: 33–44.

Troll, W. and R. Cannan, 1953. A modified photometric ninhydrin method for the analysis of amino acid composition of the soft-shell clam *Mya arenaria* in relation to salinity of the medium. *Comp. Biochem. Physiol.,* **32**: 775–783.

Tschiersch, B. and K. Mothes, 1963. Amino acids: structure and distribution. Pages 1–90 in M. Florkin and H. S. Mason, Eds., *Comparative biochemistry, Vol. V.* Academic Press, London, New York.

INDEX

A

Abdominal rotation, in mosquito, 283
Abercrombie, R. and P. De Weer. The electric current generated by the sodium pump of squid giant axon: effects of external potassium and internal ADP, 398
Absorption kinetics of gut, in *Opsanus tau*, 399
Acclimation, and molting, in lobsters, 538
Acetylcholine bioassay, in frog sartorius nerve-muscle, 417
Ache, B. W., Z. M. Fuzessery and W. E. S. Carr. Antennular chemosensitivity in the spiny lobster, *Panulirus argus:* comparative tests of high and low molecular weight stimulants, 273
Actin-binding protein, in chicken smooth muscle, 409
Actin localization, by meromyosin and fluorescence microscopy, 413
Adaptation, in sea anemones, 478
  to thermal stress, in *Fundulus*, 548
Adrenalin, effect on teleost melanophore innervation, 414
Adrenergic vesicles, 428
Aedes aegypti, terminalial inversion in, 283
Aequipecten irradians, hinge ligament in, 161
Afferent activity, in toadfish labyrinth, 414
Age of maturity, heritability of, in *Eurytemora herdmani*, 200
Albumin, effect on synaptic transmission at frog sartorius neuromuscular junction, 430
  requirements, and temperature, in *Artemia*, 314
Allen, N. S., R. D. Allen and T. E. Reinhart. Confirmed: the existence of abundant endoplasmic filaments in *Nitella*, 398
Allen, R. D. See N. S. Allen, 398
Allozymes, in *Modiolus demissus*, 404
Amino acids, action of osmium tetroxide on, 418
Amino acid analysis, of bivalve ligament and shell protein, 161
Amino-triazole, cytotoxicity of, 370
Amitrole, cytotoxic effects on *Daphnia*, 370
Ammonia, effects on growth rate, in snails, 386
  and urease, in lugworm, 96
AMP requirements, and temperature, in *Artemia*, 314
Amphibian oocytes, maturational changes in, 429
Anacystis nidulans, phycobiliproteins in, 405
Anderson, O. R. and A. W. H. Bé. A cytochemical fine structure study of phagotrophy in a planktonic foraminifer, *Hastigerina pelagica* (d'Orbigny), 437
Anglerfish islets, somatostatin biosynthesis in, 422
Annelida, sodium and chloride exchange in, 587
Anomia achaeus, function of gills in, 331
Antarctic cod, plasma protein synthesis in, 414
Anthozoa, genetics and asexual reproduction in, 478
Aphidicolin, effects on sea urchin embryos, 419
Aposymbiotic hydra, production of, 225
Arbacia, fertilization protease of, 406
  histone genes, ultrastructure of, 435
  micromeres from, 427
  nicotinamide-induced cleavage arrest in, 416
Arbacia eggs, and embryos, mRNA in, 410
  fertilization membrane, effects of procaine and tetracaine on, 412
Arbacia embryos, echinochrome oxidation in, 423
  effect of aphidicolin on, 419
  repetitive sequences in, RNA of, 410
Arenicola cristata, urease from, 96
Armstrong, P. B. Interaction of motile blood cells of *Limulus in vitro*, 399
Arsenazo III, fusion of lipid vesicles using, 407
Artemia, as food for foraminifera, 437
  effects of temperature on nutrition in, 314
Ascidian embryos, melanogenesis in, 434
Astacus leptodactylus, hemolymph coagulation in, 467
Astropecten scoparius, metamorphosis and development in, 560
ATP-ase, in synaptic vesicles of frog sartorius neuromuscular junction, 426
Atypic muscles, associated with terminalia rotation, in mosquitoes, 283
Axon, squid, voltage clamp currents from, 430
  and Fourier transform, 425

B

Babiarz, P. See P. Dunham, 407
Bacterial physiology, 574
Balanus, photoreceptor, regeneration in presynaptic terminal of, 427
Balanus cariosus, and *B. hameri*, central neurons in, 141
Balanus nubilus ganglion, use of potential sensitive dye on, 411
Ball, E. G. Pigments in the eggs of *Limulus polphemus*, 399
Baran, M. M. See C. M. Cheney, 405
Barkalow, D. and A. Farmanfarmaian. Unstirred layers—their effect on intestinal absorption kinetics for glycine and on sodium-glycine interactions in the marine teleost, *Opsanus tau*, 399
Barker, J. L. and H. B. Pollard. Calcium directly regulates transmitter uptake by squid brain synaptosomes, 400
Barlow, R. B. Jr., S. J. Bolanowski, Jr. and M. L. Brachman. Efferent mediated circadian changes in the neural activity of the *Limulus* lateral eye, 400
Barnacle, central neurons in, 141
  ganglion, use of potential sensitive dye on, 411
  photoreceptor, regeneration in presynaptic terminal of, 427
Barton, S. Comparison of facilitation and delayed release at the frog neuromuscular junction, 401
Bauer, G. E. See B. D. Noe, 422
Bayne, B. L. See A. J. Becker, Jr., 401
Bé, A. W. H. See O. R. Anderson, 437
Beale, S. I. See M. Mishkind, 420
Becker, A. J., Jr. and B. L. Bayne. Aspects of the respiratory response of *Carcinus maenas* (L.) to hypoxia and H_2S exposure, 401
Behavior, gastropod larvae, 182
  of polyps and medusae of *Podocoryne selena*, 214
  of *Renilla*, 518
  responses of larval crustaceans, 126
Biometric analyses, of heritability, in copepods, 200
Biomphalaria glabrata, chemical ecology of, 386
Bioradiation, in *Gonyaulax*, 427
  in luminous bacteria, 428
Biosynthesis, somatostatin, in anglerfish islets, 422
Bishop, S. H. See L. Cooley, 96
Bivalve gills, function of, in suspension feeding, 331
Bivalve molluscs, hinge ligament in, 161
Blastomeres of *Spisula solidissima* embryos, RNA in, 405
Blitz, A. See A. Previte, 426
Blood cells, of *Limulus*, 399
Bloodgood, R. A. and J. L. Rosenbaum. Ultrastructural observations on the squid giant axon and associated Schwann cell sheath, 402
Blood pressure, and locomotion, in bluefish, 407
Bluefish, blood pressure and locomotion in, 407
Blue-green algae, epizoic on crustacea, 489
Body size, and oxygen uptake, in tunicates, 297
  heritability of, in *Eurytemora herdmani*, 200
Bolanowski, S. J., Jr. See R. B. Barlow, Jr., 400
Borisy, G. G. and S. Inoué. *In vitro* studies on the physiology of mitosis in oocytes of *Chaetopterus*, 402
Brachiolaria stages, lacking in *Astropecten*, 560
Brachman, M. L. See R. B. Barlow, Jr., 400
Brain synaptosomes, squid, transmitter uptake by, 400
Branchial sac, and oxygen uptake, in tunicates, 297
Braun, B., B. W. Nagle and L. Kedes. Histone messenger RNA expression in echinoid hybrid embryos, 403
Bremner, T. A. See A. J. Rosenspire, 427
Bruner-Lorand, J. See L. Lorand, 419
Bryozoans, cheilostome, Gompertzian growth in, 416
Bursting neurons, barnacle, effects of depolarizing and hyperpolarizing currents and high Mg^{+2}, low Ca^{+2} concentrations, 141
Burzio, L. O. See S. S. Koide, 416

C

Calcium, and light adaptation, in photoreceptors of *Limulus*, 405
  as regulator for transmitter uptake, in squid brain synaptosomes, 400
  binding sites, in synaptic vesicles of toadfish sonic muscle, 412
Cape Cod ecosystems, respiration of, 424
Carbohydrates, histochemistry of, in sea star gonad, 357
Carcinus maenas, respiratory response of, 401
Carnivorous diet, of planktonic foraminifera, 437

Carr, W. E. S. See B. W. Ache, 273

Catapane, E. J. A pharmacological and electrophysiological study of ciliary innervation of the gill of *Mytilus edulis* (Bivalvia), 403

Catfish, response in electric fields, 415
  spatial discrimination in electric fields, 415

Central neurons, rhythmical patterned outputs in barnacles, 141

Cercaria, cystophorous, from *Nassarius trivittatus,* 433

Chaetopterus, mitosis in oocytes of, 402

Chaisson, R. E., L. A. Serunian and T. J. M. Schopf. Allozyme variation between two marshes and possible heterozygote superiority within a marsh in the bivalve *Modiolus demissus,* 404

Chang, D. C. Pulsed nuclear magnetic resonance studies of squid giant axon, 404

Chappell, R. L. See J. A. Patterson, 423

Charlton, J. S. and A. Fein. Are intracellular calcium ion injections equivalent to light adaptation in the ventral photoreceptors of *Limulus?,* 405

Cheilostome bryozoans, Gompertzian growth in, 416

Chemical composition, of hinge ligament, in bivalve molluscs, 161
  ecology, of *Biomphalaria glabrata,* 386

Chemosensitivity, antennular, in lobsters, 273

Cheney, C. M., M. M. Baran and J. V. Ruderman. Separation of blastomeres of *Spisula solidissima* embryos and comparison of RNA and protein of the isolated blastomeres, 405

Chevone, B. I. and A. G. Richards. Ultrastructure of the atypic muscles associated with terminalial inversion in male *Aedes aegypti* (L), 283

Chicken smooth muscle, actin-binding protein in, 409

Chloride and sodium exchange, in *Nereis,* 587

Chromaffin granules and laser light scattering, 428

Cilia, control by nerves, in gastropod larvae, 182
  in bivalve gills, 331

Ciliary innervation, and serotonin, in *Mytilus edulis,* 403

Ciliogenesis, in sea urchin embryos, protein synthesis during, 432

Ciona intestinalis, melanogenesis in, 434

Circadian changes, in *Limulus* lateral eye, 400

Circadian rhythm, in corals, 236

Cirripedia, shadow reflex in, 141

Cladocera, cytotoxic effect of herbicides on, 370

Cnidaria, genetics and asexual reproduction in, 478

Coagulation, of hemolymph, in crayfish, 467

Cohen, L. B. See A. Grinvald, 411

Collagenase digestion, effect on post-synaptic membrane of frog muscle, 430

Contact behavior, of *Limulus* blood cells, 399

Cooley, L., D. Crawford and S. H. Bishop. Urease from the lugworm, *Arenicola cristata,* 96

Copepod, marine, heritability of traits in, 200

Coral, circadian rhythm in, 236
  gorgonian, feeding in, 344

Cosner, J. C. and R. F. Troxler. Phycobiliprotein synthesis in protoplasts of the unicellular cyanophyte, *Anacystis nidulans,* 405

Crassostrea, function of gills in, 331
  hinge ligament in, 161

Crawford, D. See L. Cooley, 96

Crayfish, hemolymph coagulation in, 467

Crustacea, chemoreception in, 273
  cytotoxic effect of herbicides on, 370
  nutrition and effects of temperature on, 314
  larvae, shadow response in, 126

Csernansky, J. G., E. S. Harris, R. Lifset, A. Grossman, W. Troll and M. Levy. "Fertilization product" protease of *Arbacia punctulata*: Ca^{++} effects, protein and synthetic substrates, 406

Ctenophore predation, on larval crustaceans, 126

Currents, voltage clamp, from squid giant axon, 430

Cyanophytes, phycobiliproteins in, 405

Cytotoxicity, of amino-triazole, 370

D

Damassa, D. A., T. L. Davis, D. M. Shotton, J. E. Heuser, D. Pumplin and T. S. Reese. Structure of nerve terminals in frog muscle after prolonged treatment with brown widow spider venom, 406

Dangott, L. J. See J. K. Haynes, 413

Daphnia, diel feeding rhythm of, 433

Daphnia pulex, effect of herbicides on, 370

Davis, T. L. See D. A. Damassa, 406

Dendraster excentricus, suspension feeding in, 247

Development, of *Astropecten scoparius,* 560

De Weer, P. See R. Abercrombie, 398

Diatoms, reproductive strategy of, 421

Dicumarol, effect on electrical properties of lobster muscle fibers, 421

Diel feeding rhythm, of *Daphnia,* 433

Diet, of *Dendraster excentricus,* 247

Digestion, in *Histriobdella,* 489

Dispersion patterns, of spionid polychaetes, 433

Diurnal variation of photosynthesis in *Ulva,* 420

Dogfish, tryptophan photoproducts, 436
  brain tubulin, helical ribbons in, 417

Dopamine, effect on teleost melanophore innervation, 414

Dragonfly median ocellus, receptor cells, 423

DuBois, A. B., R. S. Fox, A. N. Wilner and R. H. Lambertsen. Blood pressure and locomotion in bluefish (*Pomatomus saltatrix*), 407

Dunham, P., P. Babiarz, A. Israel, A. Zerial and G. Weissmann. A general method for the study of fusion of lipid vesicles using a calcium-sensitive dye, arsenazo III, 407

Durand, J. P. Ocular development and involution in the European cave salamander, *Proteus anguinus* Laurenti, 450

Durliat, M. and R. Vranckx. Coagulation in the crayfish, *Astacus leptodactylus*: attempts to identify a fibrinogen-like factor in the hemolymph, 467

E

Echinochrome oxidation, in *Arbacia* embryos, 423

Echinoid embryos, mRNA in, 403

Ecology, chemical, of *Biomphalaria glabrata,* 386

Ecosystems, Cape Cod, respiration of, 424

Eel, ganglion cells, retinal spatial interactions in, 429
  retina, ERG analysis of receptor types and photopigments in, 411

Eggs, pigments in, of *Limulus,* 399

Electric current, by sodium pump in squid giant axon, 398

Electric fields, response of catfish in, 415

Electrical properties of lobster muscle fibers, effect of metabolic inhibitors on, 421

Electron microscopy, of *Daphnia,* 370
  of foraminifera, 437
  of histone genes, in sea urchin, 435
  of *Histriobdella homari,* 489
  of muscles, in *Aedes aegypti,* 283
  of *Nitella* endoplasmic filaments, 398
  of Schwann cell sheath, in squid giant axon, 402
  scanning, of mouse eggs, 435

Electrophysiology, in chemoreception study, 273

Ellison, R. P. See S. S. Koide, 416

Elongation factor 1, in toadfish liver, 422

Elvin, D. W. Seasonal growth and reproduction of an intertidal sponge, *Haliclona permollis* (Bowerbank), 108
  Observations on the effects of light and temperature on the apparent neurosecretory cells of *Mytilus edulis,* 408

Embryos, ascidian, melanogenesis in, 434
  echinoid, mRNA in, 403
  sea urchin, echinochrome oxidation in, 423
  sea urchin, effect of aphidicolin on, 419
  sea urchin, protein synthesis during ciliogenesis and regeneration, 432
  Spisula solidissima, RNA in blastomeres of, 405

Embryonic development, and ^{3}H-TPP, 260

Endoplasmic filaments, in *Nitella,* 398

Enos, R. See L. C. U. Junqueira, 414

Ensis directus, hinge ligament in, 161

Environment, and growth and reproduction in sponges, 108

Enzyme, ammonia-forming, in lugworm, 96

Epel, D. L. See L. Lorand, 419

ERG analysis, of receptor types and photopigments in eel retina, 411

Erythrocyte, human, transglutaminase activity in, 419
  trout, permeability to nonelectrolytes, 322

Estuarine molluscs, osmotic adjustment in, 601

Ethylene glycol, permeability of trout erythrocytes to, 322

Euphyllia rugosa, circadian rhythms in, 236

Eurytemora herdmani, heritability of traits in, 200

Eyes, rudimentary, in *Proteus,* 450

F

Facilitation, at frog neuromuscular junction, 401

Farmanfarmaian, A. See D. Barkalow, 399

Feeding, in *Histriobdella,* 489
  in *Leptogorgia,* 344
  in *Renilla,* 518
  in sand dollars, 247
  rhythm, diel, of *Daphnia,* 433
  stimuli, the role of macromolecules as, in lobster chemoreception, 273

Fein, A. See J. S. Charlton, 405

Fertilization membrane, sea urchin egg, effects of procaine and tetracaine on, 412

Fertilization protease, of *Arbacia,* 406

Fisher, F. M., Jr. See G. A. Kahler, 161

Fisher, T. R. Oxygen uptake of the solitary tunicate *Styela plicata,* 297

Fishman, H. M., L. E. Moore and D. J. M.

Poussart. Identification of Na conduction noise in squid axon, 408
Fishman, H. M. See D. Poussart, 425
Flecha, M. See R. Marco, 420
Fluorescence microscopy, for actin localization, 413
Food web, of salt marshes, 418
Foraminifera, nutrition and phagotrophy in, 437
Forward, R. B., Jr. A shadow response in a larval crustacean, 126
Fourier transform, and squid axon, 425
Fowler, W. E. and T. D. Pollard. Purification and characterization of smooth muscle actin-binding protein, 409
Fox, R. S. See A. B. DuBois, 407
Free amino acids, in *Urosalpinx*, 601
Fregien, N., M. Marchionni and L. Kedes. Histone gene organization of six marine invertebrates, 409
French, C. and T. Humphreys. Repetitive sequence representation in sea urchin embryo nuclear RNA, 410
Frog, myocardium, ion content of, 306
  ganglion cells, retinal spatial interactions in, 429
  muscle, synaptic vesicles in, after treatment with brown widow spider venom, 406
  muscle postsynaptic membrane, ultrastructure after collagenase digestion, 430
  neuromuscular junction, effect of isethionate on, 425
  neuromuscular junction, facilitation in, 401
  neuromuscular junction synaptic transmission, and substance P, 432
  sartorius nerve-muscle, acetycholine bioassay in, 417
  sartorius neuromuscular junction, effect of albumin on synaptic transmission of, 430
  sartorius neuromuscular junction, effect of ATP-ase on synaptic vesicles of, 426
Frontal cilia, in bivalve gills, 331
Fundulus heteroclitus, adaptation to thermal stress in, 548
  isohemoglobins from, 413
Fungiidae, circadian rhythm in, 236
Fuzessery, Z. M. See B. W. Ache, 273
Fyrberg, E. A. and J. V. Ruderman. Investigation of a poly(A) minus nonhistone messenger RNA in sea urchin eggs and embryos, 410

G

Gametogenesis, in sponges, 108
Ganglion, barnacle, use of potential sensitive dye on, 411
Gas embolism, in teleosts, 417
Gastropod larvae, ciliary activity in, 182
Gastropoda, osmotic adjustment in, 601
Gelder, S. R. See J. B. Jennings, 489
Genetics and asexual reproduction, of *Metridium senile*, 478
Genitalia, rotation of, in mosquito, 283
Giant axon, squid, nuclear magnetic resonance in, 404
  Schwann cell sheath, 402
  sodium pump of, 398
Glycerol, permeability of trout erythrocytes to, 322
Gompertzian growth, in cheilostome bryozoans, 416
Gonad, sea star, nutrient reserves of, 357
Gonyaulax, bioradiation in, 427
Gordon, J., R. Shapley and E. Kaplan. Receptor types and photopigments in the eel retina: an ERG analysis, 411
Gordon, J. See R. Shapley, 429
Green, D. J. See D. B. Sattelle, 428
Grinvald, A., B. M. Salzberg, L. B. Cohen, K. Kamino, A. S. Waggoner, C. H. Wang and D. Ti. Simultaneous recording from twelve neurons in the supraesophageal ganglion of *Balanus nubilus* using a new potential sensitive dye, 411
Grossman, A. See J. G. Csernansky, 406
Growth, effect of ammonia on, in snails, 386
  of sponges, 108
  rates in cheilostome bryozoans, 416
Gwilliam, G. F. The mechanism of the shadow reflex in Cirripedia III. Rhythmical patterned activity in central neurons and its modulation by shadows, 141

H

Haliclona permollis, growth and reproduction, 108
Hall, C. A. S. See B. J. Peterson, 424
Hamkalo, B. See D. Zdunski, 435
Haney, J. F. See J. L. Tiwari, 433
Hanna, R. B. and G. D. Pappas. Tetanic stimulation depresses the occurrence of Ca^{++} binding sites in synaptic vesicles of toadfish sonic muscle, 412
Hardy, C. J. See E. G. Ruby, 428
Harris, E. S., H. Schuel and W. Troll. Inhibition of fertilization membrane elevation in sea urchin eggs by procaine and tetracaine, 412
Harris, E. S. See J. G. Csernansky, 406
Harza, T. and L. Mátyás. Seasonal variations of sodium and potassium concentrations in different parts of the frog myocardium, 306

Haschemeyer, A. E. V., M. A. K. Smith and R. W. Mathews. Toadfish liver protein synthetic parameters at high doses of leucine *in vivo,* 412

Hastigerina pelagica, phagotrophy in, 437

Haynes, J. K., D. R. Wilson, M. Price, L. J. Dangott, P. Mied and D. A. Powers. Structural and functional studies of the isohemoglobins from *Fundulus heteroclitus,* 413

Helical ribbons, in dogfish brain tubulin, 417

Hemolymph coagulation, in crayfish, 467

Herbicides, effect on aquatic invertebrates, 370

Heritability, of traits in marine copepods, 200

Herman, I. M. and T. D. Pollard. Preparation of tetramethylrhodamine-heavy meromyosin for actin localization by fluorescence microscopy, 413

Hermit crabs, symbiotic with hydroids, 214

Hernandorena, A. Effects of temperature on the nutritional requirements of *Artemia salina* (L.), 314

Heuser, J. E. See D. A. Damassa, 406; D. M. Shotton, 430

Highstein, S. M. and A. L. Politoff. Modulation of afferent activity of the posterior semicircular canal of the toadfish (*Opsanus tau*) by efferent stimulation, 414

Hinge ligament, in bivalve molluscs, 161

Histone genes, sea urchin, 420
  in sea urchin, ultrastructure of, 435
  in *Spisula solidissima,* 431
  organization, of marine invertebrates, 409
  repeats, in sea urchin, 431

Histriobdella, feeding, proboscis structure, digestion, 489

Hoffmann, R. J. Genetics and asexual reproduction of the sea anemone *Metridium senile,* 478

Homarus americanus, molting and acclimation in, 538
  muscle fibers, effects of metabolic inhibitors on electrical properties of, 421

^{3}H-thymidine triphosphate, and embryonic development, 260

Hudson, A. P. Comparative studies of plasma protein synthesis in temperate and Antarctic fishes, 414

Human erythrocytes, transglutaminase activity in, 419

Humphreys, T. See C. French, 410

Hunter, F. R. Permeability of trout erythrocytes to nonelectrolytes, 322

Hydra-algae symbiosis and aposymbiotic hydra, 225

Hydra viridis, Florida strain and English strain, production of aposymbionts, 225

Hydractinia echinata and *Podocoryne selena,* 214

Hydrogen sulphide and respiratory response, in *Carcinus maenas,* 401

Hydroid, ultrastructure of, 214

Hypoxia, and respiratory response, in *Carcinus maenas,* 401

I

Ictalurus nebulosus, spatial descrimination in electric fields, 415

Image intensifier, in bioradiation studies, 427

Immunological studies of coagulation, in crayfish, 467

Inorganic ions, in *Urosalpinx,* 601

Inoué, S. See G. G. Borisy, 402

Insemination, and ultrastructure, of mouse eggs, 435

Intermolt cycle, in lobster, 538

Intertidal sponge, growth and reproduction in, 108

Inversion, terminalial, in mosquito, 283

Involution, in *Proteus,* 450

Ion content, in frog myocardium, 306

Isethionate, effect on isolated secretory vesicles and frog neuromuscular junction, 425

Isohemoglobins, from *Fundulus heteroclitus,* 413

Israel, A. See P. Dunham, 407

J

Jennings, J. B. and S. R. Gelder. Observations on the feeding mechanism, diet and digestive physiology of *Histriobdella homari* van Beneden 1858: an aberrant polychaete symbiotic with North American and European lobsters, 489

Jørgensen, C. B. Comparative studies on the function of gills in suspension feeding bivalves, with special reference to effects of serotonin, 331

Junqueira, L. C. U., R. Enos and F. Reinach. Effect of 6-OH-dopamine, 5-OH-dopamine and adrenalin on teleost melanophore innervation and intracytoplasmatic microfilaments, 414

Juxtamusium maldivense, function of gills in, 331

K

Kahler, G. A., F. M. Fisher, Jr. and R. L. Sass. The chemical composition and mechanical properties of the hinge ligament in bivalve molluscs, 161

Kalmijn, A. J., C. A. Kolba and V. Kalmijn. Orientation of catfish (*Ictalurus nebulosus*) in strictly uniform electric fields: I. Sensitivity of response, 415

Kalmijn, A. J. See V. Kalmijn, 415

Kalmijn, V., C. A. Kolba and A. J. Kalmijn. Orientation of catfish (*Ictalurus nebulosus*) in strictly uniform electric fields: II. Spatial discrimination, 415

Kalmijn, V. See A. J. Kalmijn, 415

Kamino, K. See A. Grinvald, 411

Kano, Y. T. See C. Oguro, 560

Kaplan, E. See J. Gordon, 411

Kastendiek, J. Behavior of the sea pansy *Renilla kollikeri* Pfeffer (Coelenterata: Pennatulacea) and its influence on the distribution and biological interactions of the species, 518

Kaufman, H. W. See J. C. Lisak, 418

Kaufmann, K. W. Gompertzian growth in cheilostome bryozoans, 416

Kedes, L. H. See B. Braun, 403; N. Fregien, 409; R. Marco, 420; D. J. States, 431; D. Zdunski, 435

Kennedy, J. R. See T. W. Schultz, 370

Kiehart, D. P. See M. M. Pratt, 426

Koehn, R. K. See J. B. Mitton, 548

Koide, S. L. See S. S. Koide, 416

Koide, S. S., R. P. Ellison, L. O. Burzio and S. L. Koide. Demonstration of poly (adenosine diphosphate ribose) synthase activity during early sea urchin development and investigation of nicotinamide-induced cleavage arrest, 416

Koide, S. S. See M. A. Lorand, 419

Kolba, C. A. See A. J. Kalmijn, 415; V. Kalmijn, 415

Komatsu, M. See C. Oguro, 560

Kriebel, M. E., D. R. Matteson and G. D. Pappas. Bioassay studies of acetylcholine in the stimulated frog sartorius nerve-muscle preparation and following denervation, 417

L

Lambertsen, R. H. Acute thermal gradients as a possible cause of gas embolism and associated mortality in teleosts, 417

Lambertsen, R. H. See A. B. DuBois, 407

Langford, G. M. The formation of helical ribbons during *in vitro* polymerization of tubulin from dogfish brain, 417

Langley, K. H. See D. B. Sattelle, 428

Larvae, gastropod, ciliary activity in, 182

Larval crustacean, shadow response in, 126

Laser light scattering, and chromaffin granules, 428

Lateral cilia, in bivalve gills, 331

Lateral eye of *Limulus*, circadian changes, 400

Latero-frontal cirri, in bivalve gills, 331

Lee, J. J., J. H. Tietjen, C. Mastropaolo and M. Lee. Biological factors which effect the structure and functioning of salt marsh Aufwuchs microfloral and meiofaunal assemblages, 418

Lee, M. See J. J. Lee, 418

Leptogorgia virgulata, feeding and current flow in, 344

Leucine, effect on toadfish liver protein synthetic parameters, 412
  transport and temperature, in toadfish liver, 424

Leversee, G. J. Flow and feeding in fan-shaped colonies of the gorgonian coral, *Leptogorgia*, 344

Levy, M. See J. G. Csernansky, 406

Lifset, R. See J. G. Csernansky, 406

Light, adaptation in photoreceptors of *Limulus*, 405
  and temperature, effects on *Mytilus edulis* neurosecretory cells, 408
  response in larval crustaceans, 126

Limulus, blood cells of, 399
  circadian changes in lateral eye of, 400
  pigments in eggs of, 399
  photoreceptors of, light adaptation in, 405

Lipids, histochemistry of, in sea star gonad, 357

Lipid vesicles, fusion of, using arsenazo III, 407

Lisak, J. C., H. W. Kaufman, P. Maupin-Szamier and T. D. Pollard. The action of osmium tetroxide on proteins and amino acids, 418

Lobster, antennular chemosensitivity in, 273
  molting and acclimation in, 538
  symbiotes of, 489
  muscle fibers, effect of metabolic inhibitors on electrical properties of, 421

Localization, of translatable RNA, in *Xenopus laevis*, 424

Locomotion and blood pressure, in bluefish, 407

Lodge, R. See J. D. Thomas, 386

Loligo, axon and Fourier transform, 425
  giant axons, voltage clamp currents from, 430
  nuclear magnetic resonance in giant axon, 404
  sodium conduction in axon of, 408
  sodium pump, of giant axon, 398

Lorand, L., L. B. Weissmann, J. Bruner-Lorand and D. L. Epel. Role of intracellular transglutaminase in the Ca^{2+}-

mediated cross-linking of erythrocyte membrane proteins, 419
Lorand, M. A., S. J. Segal and S. S. Koide. Effects of the anti-viral agent Aphidicolin on early development of sea urchin embryos, 419
Lugworm, urease from, 96
Luminous bacteria, bioradiation in, 428
of pinecone fish, 574
Lysosomes, cytochemistry of, in phagocytosis and digestion, in *Hastigerina*, 437
Lytechinus pictus, histone genes, 420

M

Mackie, G. O., C. L. Singla, and C. Thiriot-Quievreux. Nervous control of ciliary activity in gastropod larvae, 182
Madreporic plate, in development of *Astropecten,* 560
Magnetic resonance, nuclear, in squid giant axon, 404
Mangelia nebula, morphology and nervous control of ciliary action in, 182
Marchionni, M. See N. Fregien, 409
Marco, R., M. Flecha, and L. H. Kedes. Studies on the organization and purification of sea urchin (*L. pictus*) histone genes, 420
Marine bacteria, symbiosis with pinecone fish, 574
Marine fish, adaptation to thermal stress in, 548
Marine invertebrates, histone gene organization of, 409
Mastroianni, L., Jr. See D. P. Wolf, 435
Mastropaolo, C. See J. J. Lee, 418
Mathews, R. W. See A. E. V. Haschemeyer, 412
Matteson, D. R. See M. E. Kriebel, 417
Maturational changes, in amphibian oocytes, 429
Mátyás, L. See T. Harza, 306
Maupin-Szamier, P. See J. C. Lisak, 418
Mauzerall, D. See M. Mishkind, 420
McCarthy, J. F., A. N. Sastry, and G. C. Tremblay. Thermal compensation in protein and RNA synthesis during the intermolt cycle of the American lobster, *Homarus americanus,* 538
McLaren, I. A. Inheritance of demographic and production parameters in the marine copepod *Eurytemora herdmani,* 200
Mechanics, of hinge ligament, in bivalve molluscs, 161
Median ocellus, dragonfly, receptor cells, 423
Meiofaunal and microfloral assemblages, salt marsh Aufwuchs, 418
Melanogenesis, in ascidian embryos, 434
Melanophore innervation, teleost, effect of dopamine and adrenalin on, 414
Membrane fusion, using arsenazo III, 407
Mercenaria mercenaria, hinge ligament in, 161
Meromyosin, for actin localization by fluorescence microscopy, 413
Merrifield, P. A. See R. E. Steele, 431
Metamorphosing bipinnaria, in *Astropecten,* 560
Metamorphosis, in sea-star, 560
Metridium senile, genetics and asexual reproduction of, 478
Microfloral and meiofaunal assemblages, salt marsh Aufwuchs, 418
Micromeres, from sea urchin, 427
Mied, P. See J. K. Haynes, 413
Mills, C. E. *Podocoryne selena,* a new species of hydroid from the Gulf of Mexico, and a comparison with *Hydractinia echinata,* 214
Mishkind, M., D. Mauzerall and S. I. Beale. Diurnal variation *in situ* of photosynthetic capacity and efficiency in *Ulva,* 420
Mitosis, in oocytes, of *Chaetopterus,* 402
Mitton, J. B. and R. K. Koehn. Morphological adaptation to thermal stress in a marine fish, Fundulus heteroclitus, 548
Modiolus demissus, allozymes in, 404
Molluscs, bivalve, hinge ligaments in, 161
Molting, and acclimation, in lobster, 538
Monocentris japonica, luminous bacteria of, 574
Moody, W., Jr. Effects of metabolic inhibitors on the electrical properties of lobster muscle fibers, 421
Moore, L. E. See H. M. Fishman, 408; D. Poussart, 425
Mooseker, M. S. See M. M. Pratt, 426
Morphological adaptation, to thermal stress, in *Fundulus heteroclitus,* 548
Mosquito, terminalial inversion in, 283
Mouse eggs, ultrastructure of, 435
mRNA, in sea urchin eggs and embryos, 403, 410
Muratore, J. F. One of the consequences of being a diatom: a simulation analysis of the reproductive strategy of diatoms, 421
Muricidae, osmotic adjustment in, 601
Muscles and terminalial inversion, in mosquito, 283
Muscular organization, gastropod larvae, 182
Mutualism, of luminous bacteria and pinecone fish, 574
Mya arenaria, hinge ligament in, 161

Myocardium, frog, ion content of, 306
Myofibrils, from skeletal muscle, 426
Mytilus edulis, hinge ligament in, 161
  serotonin and ciliary innervation in, 403
  neurosecretory cells, effects of light and temperature on, 408

N

Nagle, B. W. See B. Braun, 403
Nassarius trivittatus, cystophorous cercaria from, 433
Nealson, K. H. See E. G. Ruby, 574
Nereis, sodium and chloride exchange in, 587
Nervous organization, of gastropod larvae, 182
Neuromuscular junction of frog, facilitation at, 401
  synaptic transmission, and substance P, 432
Neurophysiological correlates of behavior, in barnacles, 141
Neurosecretory cells, of *Mytilus edulis*, effects of light and temperature on, 408
Nicosia, S. V. See D. P. Wolf, 435
Nicotinamide-induced cleavage arrest, in sea urchin, 416
Nielsen, J. B. K. Toadfish liver elongation factor 1: time course of cold acclimation and hormonal effects, 422
Nimitz, M. A. Histochemical changes in gonadal nutrient reserves correlated with nutrition in the sea stars, *Pisaster ochraceus* and *Patiria miniata*, 357
Nitella, endoplasmic filaments in, 398
Nitrogen metabolism, invertebrate, 96
Noe, B. D., G. C. Weir and G. E. Bauer. Somatostatin biosynthesis in anglerfish islets, 422
Nonbrachiolarian type of development, in asteroids, 560
Nonelectrolytes, permeability of trout erythrocytes to, 322
Nuclear uptake, and ^{3}H-TPP utilization in embryonic development, 260
Nutrient reserves, in sea star gonad, 357
Nutrition, effects of temperature on, in *Artemia*, 314
  of *Histriobdella*, 489

O

Ocular development, in *Proteus*, 450
Oguro, C., M. Komatsu and Y. T. Kano. Development and metamorphosis of the sea-star, *Astropecten scoparius* Valenciennes, 560
Oocytes, amphibian, maturational changes in, 429
  of *Chaetopterus*, mitosis in, 402
Opsanus tau, absorption kinetics of gut, 399
  afferent activity in labyrinth of, 414
  leucine transport and temperature in liver of, 424
  plasma protein synthesis in, 414
  secretion of inert gas into swimbladder of, 434
  sonic muscle, calcium binding sites in synaptic vesicles of, 412
Osmium tetroxide, action of, on proteins and amino acids, 418
Osmotic adjustment, in *Urosalpinx*, 601
Oxygen uptake, in *Styela plicata*, 297

P

Panulirus argus, antennular chemosensitivity in, 273
Pappas, G. D. See R. B. Hanna, 412; M. E. Kriebel, 417
Pardy, R. L. The production of aposymbiotic hydra by the photodestruction of green hydra zoochlorellae, 225
Particle retention, in bivalve gills, 331
Patiria miniata, nutrition in, 357
Patterson, J. A. and R. L. Chappell. Electrical activity and structure of receptor and second-order cells of the median ocellus of the dragonfly, 423
Pazoles, C. See H. B. Pollard, 425
Pectinaria gouldii, embryonic development of, 260
Permeability, of trout erythrocytes, 322
Perry, G. Echinochrome oxidation in *Arbacia punctulata* embryos, 423
Persell, R. Temperature dependency of leucine transport by toadfish liver *in vivo*, 424
Peterson, B. J., C. A. S. Hall, J. P. Reed and T. Wood. Comparative respiration of Cape Cod ecosystems, 424
Peterson, B. J. See J. L. Tiwari, 433
Phagotrophy, in foraminifera, 437
Phillips, C., J. Ruderman and M. Rosbash. Regional localization of translatable RNA in *Xenopus laevis*, 424
Phosphohexose-isomerase variation, in *Metridium*, 478
Photobacterium fischeri, symbiotic association with *Monocentris japonica*, 574
Photodestruction, of symbionts, and aposymbiont formation, 225
Photopigments, and receptor types in eel retina, ERG analysis of, 411
Photoproducts, tryptophan, in dogfish, 436
Photoreceptor, barnacle, regeneration in presynaptic terminal of, 427
  Limulus, light adaptation in, 405

Photosynthesis, diurnal variation of, in *Ulva*, 420
Phototaxis, in larval crustaceans, 126
Phycobiliproteins, in *Anacystis nidulans*, 405
Pigments, in eggs, of *Limulus*, 399
Pisaster ochraceus, nutrition in, 357
Placopecten magellanicus, hinge ligament in, 161
Planktonic foraminfera, phagotrophy in, 437
Plasma hemocytes, free, in crayfish, 467
Plasma protein synthesis, in fish, 414
Plasmatic clottable protein, in *Astacus*, 467
Pneumoderma atanticum, nervous control of ciliary action in, 182
Podocoryne selena, new species of hydroid, 214
Pogell, B. M. See A. J. Rosenspire, 427
Politoff, A. L. See S. M. Highstein, 414; A. Previte, 426; L. M. Spindel, 430
Pollard, H. B., A. Steinacker and C. Pazoles. Isethionate blocks release of transmitter from isolated secretory vesicles and from frog neuromuscular junction, 425
Pollard, H. B. See J. L. Barker, 400
Pollard, T. D. See W. E. Fowler, 409; I. M. Herman, 413; J. C. Lisak, 418
Polychaetes, sodium and chloride exchange in, 587
  spionid, dispersion patterns of, 433
  symbiotic, 489
  embryos, use of ^{3}H-TTP by, 260
Polymorphism, in sea anemones, 478
Pomatomus saltatrix, blood pressure and locomotion in, 407
Population genetics, of sea anemone, 478
Post-synaptic potentials, in barnacle central neurons, 141
Potassium, in frog myocardium, 306
Poussart, D., L. E. Moore and H. M. Fishman. Maximally-fast measurement of complex admittance of squid axon through digital Fourier transform, 425
Poussart, D. J. M. See H. M. Fishman, 408
Powers, D. A. See J. K. Haynes, 413
Powles, M. See J. D. Thomas, 386
Pratt, M. M., M. S. Mooseker, D. P. Kiehart and R. E. Stephens. Cyclic contraction and relaxation of glycerinated myofibrils isolated from skeletal muscle, 426
Presynaptic terminal, of barnacle photoreceptor, regeneration in, 427
Previte, A., S. Rose, A. Blitz and A. L. Politoff. The incorporation of an exogenous ATP-ase (apyrase) into the synaptic vesicles of the frog sartorious neuromuscular junction causes block of synaptic transmission and vesicle depletion, 426
Price, M. See J. K. Haynes, 413
Procaine, effect on sea urchin fertilization membrane, 412
Protease, fertilization, of *Arbacia*, 406
Proteins, action of osmium tetroxide on, 418
Protein synthesis, during intermolt cycle in lobster, 538
  in ciliogenesis and regeneration in sea urchin embryos, 432
  parameters of toadfish liver, effect of leucine on, 412
Proteus anguinus, rudimentary eyes in, 450
Pteria macroptera, function of gills in, 331
Pumplin, D. See D. A. Damassa, 406
Purine metabolism, invertebrate, 96
Pycnodonte, function of gills in, 331
Pyrimidine, biosynthesis *de novo* and salvage pathway, in lobster, 538

Q

Quantitative genetics, in copepods, 200

R

Rana esculenta, ion content of myocardium in, 306
Receptor cells, of dragonfly median ocellus, 423
Receptor types, and photopigments in eel retina, ERG analysis of, 411
Recoagulation variations and anticoagulants, in crayfish, 467
Reed, J. P. See B. J. Peterson, 424
Reese, T. S. See D. A. Damassa, 406; D. M. Shotton, 430
Regeneration, in presynaptic terminal of barnacle photoreceptor, 427
  in sea urchin embryos, protein synthesis during, 432
Reinach, F. See L. C. U. Junqueira, 414
Reinhart, T. E. See N. S. Allen, 398
Renilla kollikeri, behavior, 518
Repetitive sequence, in sea urchin embryo RNA, 410
Reproduction, asexual, and genetics of *Metridium*, 478
  in sea stars, 357
  of sponges, 108
  strategy of diatoms in, 421
Resonance, nuclear magnetic, in squid giant axon, 404
Respiration, of Cape Cod ecosystems, 424
Respiratory response, of *Carcinus maenas*, 401
Response of catfish in electric fields, 415

Retinal spatial interactions, in eel and frog ganglion cells, 429
Reynolds, G. T. Evidence for *in vivo* bioradiation at wavelengths greater than 6400 Å in *Gonyaulax polyedra*, 427
Reynolds, G. T. See E. G. Ruby, 428
Rhithropanopeus harrisii, shadow response in, 126
Rhizopodial capture, of prey in foraminifera, 437
Richards, A. G. See B. I. Chevone, 283
RNA, in blastomeres of *Spisula solidissima* embryos, 405
  localization of translatable, in *Xenopus laevis,* 424
  sea urchin embryo, repetitive sequence in, 410
  synthesis, during intermolt cycle in lobster, 538
Rosbash, M. See C. Phillips, 424
Rose, S. See A. Previte, 426
Rosenbaum, J. L. See R. A. Bloodgood, 402
Rosenspire, A. J., T. A. Bremner and B. M. Pogell. Expression of differentiated function in cultures of isolated sea urchin micromeres, 427
Ross, W. N. and A. E. Stuart. Regenerative activity in the presynaptic terminal region of the barnacle photoreceptor, 427
Rotational mechanism, muscles involved in terminalia inversion in mosquitoes, 283
Ruby, E. G. and K. H. Nealson. Symbiotic association of *Photobacterium fischeri* with the marine luminous fish *Monocentris japonica:* a model of symbiosis based on bacterial studies, 574
Ruby, E. G., G. T. Reynolds, A. J. Walton and C. J. Hardy. Evidence for *in vivo* bioradiation at wavelengths greater than 6400 Å in five strains of luminous bacteria, 428
Ruderman, J. V. See C. M. Cheney, 405; E. A. Fyrberg, 410; C. Phillips, 424; R. E. Steele, 431
Rudimentary eyes, in *Proteus,* 450

S

Salamander, European cave, rudimentary eyes in, 450
Salinity, effect on sodium and chloride exchange in *Nereis,* 587
Salmo clarki, S. gairdneri, S. trutta, permeability of erythrocytes in, 322
Salt marsh Aufwuchs, microfloral and meiofaunal assemblages, 418
Salvelinus fontinalis, permeability of erythrocytes in, 322
Salzberg, B. M. See A. Grinvald, 411
Samson, D. See A. W. Schuetz, 429
Sand dollars, suspension feeding in, 247
Sass, R. L. See G. A. Kahler, 161
Sastry, A. N. See J. F. McCarthy, 538
Sattelle, D. B., D. J. Green, K. H. Langley and E. W. Westhead. Size, dispersity and aggregation of isolated secretory (chromaffin) granules, 428
Scanning electron microscopy, of *Astropecten scoparius,* 560
  of hydrozoans, 214
  of post-synaptic membrane of frog muscle after collagenase digestion, 430
Schopf, T. J. M. See R. E. Chaisson, 404
Schuel, H. See E. S. Harris, 412
Schuetz, A. M. and D. Samson. Maturational changes in amphibian oocytes injected with oocyte fractions obtained from the surf clam, 429
Schultz, T. W. and J. R. Kennedy. Cytotoxic effects of the herbicide 3-amino-1,2,4-triazole on *Daphnia pulex* (Crustacea: Cladocera), 370
Schwann cell sheath, ultrastructure of, in squid giant axon, 402
Sea anemone, genetics and asexual reproduction of, 478
Sea pansy, behavior, 518
Sea-star, development and metamorphosis in, 560
Sea star gonad, nutrient reserves of, 357
Sea urchin, fertilization protease of, 406
  histone genes, 420
  histone genes, ultrastructure, 435
  histone gene repeats in, 431
  micromeres from, 427
  nicotinamide-induced cleavage arrest in, 416
Sea urchin egg, fertilization membrane, effects of procaine and tetracaine, 412
  and embryos, mRNA in, 410
Sea urchin embryos, echinochrome oxidation in, 423
  effect of aphidicolin on, 419
  protein synthesis during ciliogenesis and regeneration, 432
  repetitive sequence in RNA of, 410
Secretory vesicles, isolated neural, effect of isethionate on, 425
Segal, S. J. See M. A. Lorand, 419
Serotonin, and ciliary innervation, in *Mytilus edulis,* 403
  effects on bivalve gill cilia, 331
Serunian, L. A. See R. E. Chaisson, 404
Sex ratio, heritability of, in *Eurytemora herdmani,* 200
Shadow reflex, in Cirripedia, 141
Shadow response, in larval crustaceans, 126

SHAPLEY, R. AND J. GORDON. Retinal spatial interactions observed in responses of eel (and frog) ganglion cells, 429
SHAPLEY, R. See J. Gordon, 411
SHOTTON, D. M., J. E. HEUSER AND T. S. REESE. A new view of the postsynaptic membrane of frog muscle: scanning electron microscopy after collagenase digestion, 430
SHOTTON, D. M. See D. A. Damassa, 406
Simulation analysis, of reproductive strategy, of diatoms, 421
SINGLA, C. L. See G. O. Mackie, 182
Skeletal muscle, myofibrils from, 426
Skeletal system, of juvenile *Astropecten*, 560
SMITH, M. A. K. See A. E. V. Haschemeyer, 412
SMITH, R. I. Exchanges of sodium and chloride at low salinities by *Nereis diversicolor* (Annelida, Polychaeta), 587
Smooth muscle, chicken, actin-binding protein in, 409
Snail, growth rate, effect of ammonia on, 386
Sodium, in frog myocardium, 306
Sodium and chloride exchange, in *Nereis*, 587
Sodium conduction, in squid axon, 408
Sodium-glycine interactions, in gut of *Opsanus tau*, 399
Sodium pump, of squid giant axon, 398
Somatic growth, in sponges, 108
Somatostatin biosynthesis, in anglerfish islets, 422
Sonic muscle synaptic vesicles of toadfish, calcium binding sites in, 412
Spatial discrimination, of catfish in electric fields, 415
Spectroscope image intensifier, 428
SPINDEL, L. M. AND A. L. POLITOFF. Albumin causes inhibition of synaptic transmission at the frog sartorius neuromuscular junction, 430
Spionid polychaetes, dispersion patterns of, 433
Spisula solidissima, hinge ligament in, 161
  histone genes in, 431
  embryos, RNA in blastomeres of, 405
Sponge, growth and reproduction, 108
Squid brain synaptosomes, transmitter uptake by, 400
Squid giant axon, Schwann cell sheath of, 402
  sodium conduction in, 408
  and Fourier transform, 425
  nuclear magnetic resonance in, 404
  sodium pump of, 398
  voltage clamp currents from, 430
Starch requirements, and temperature, in *Artemia*, 314
STARZAK, M. E. AND R. J. STARZAK. Voltage clamp currents from squid giant axons clamped with their action potentials, 430
STARZAK, R. J. See M. E. Starzak, 430
STATES, D. J. AND L. H. KEDES. Heterogeneity in the sea urchin (*Strongylocentrotus purpuratus*) histone gene repeat detected by S_1 nuclease digestion of reassociated chromosomal DNA, 431
STEELE, R. E., P. A. MERRIFIELD AND J. V. RUDERMAN. Histone gene reiteration frequency in the surf clam *Spisula solidissima*, 431,
STEINACKER, A. Effect of substance P on synaptic transmission at the frog neuromuscular junction, 432
  See H. B. Pollard, 425
STEPHENS, R. E. Differential protein syntheis and utilization during ciliogenesis and regeneration in sea urchin embryos, 432
  See M. M. Pratt, 426
Strategy, reproductive, of diatoms, 421
Strongylocentrotus, histone gene repeats in, 431
STUART, A. E. See W. N. Ross, 427
STUNKARD, H. W. A new cystophorous cercaria from *Nassarius trivittatus*, 433
Styela plicata, oxygen uptake of, 297
Survivorship, heritability of, in *Eurytemora herdmani*, 200
Suspension feeding, and water currents, in *Leptogorgia*, 344
  bivalve, 331
  of sand dollars, 247
SWEENEY, B. M. Circadian rhythms in corals, particularly Fungiidae, 236
Symbiosis, of hydra and algae, 225
  of lobster and polychaete, 489
  of luminous bacteria and pinecone fish, 574
Synaptic transmission, and substance P, at neuromuscular junction of frog, 432
  at frog sartorius neuromuscular junction, effect of albumin on, 430
Synaptic vesicles, in frog muscle, after treatment with brown widow spider venom, 406
  of frog sartorius neuromuscular junction, effect of ATP-ase on, 426
  of toadfish sonic muscle, calcium binding sites in, 412

T

Teleost, gas embolism in, 417
  melanophore innervation, effect of dopamine and adrenalin on, 414
  swimbladder, secretion of inert gas into, 434

Temperature, and *Artemia* nutrition, 314
  and leucine transport, in toadfish liver, 424
  and light, effects on *Mytilus edulis* neurosecretory cells, 408
  and oxygen uptake, in tunicates, 297
  role in reproductive behavior in sponges, 108
Temporal patterns, in corals, 236
Tetracaine, effect on sea urchin fertilization membrane, 412
Thermal stress, adaptation to, in *Fundulus*, 548
  as cause of gas embolism in teleosts, 417
Thiourea, permeability of trout erythrocytes to, 322
THIRIOT-QUIEVREUX, C. See G. O. Mackie, 182
THOMAS, J. D., M. POWLES AND R. LODGE. The chemical ecology of *Biomphalaria glabrata:* the effects of ammonia on the growth rate of juvenile snails, 386
TI, D. See A. Grinvald, 411
TIETJEN, J. H. See J. J. Lee, 418
TIMKO, P. L. Sand dollars as suspension feeders: a new description of feeding in *Dendraster excentricus*, 247
TIWARI, J. L., J. F. HANEY AND B. J. PETERSON. Computer simulation of the empirically determined diel feeding rhythm of *Daphnia*, 433
Toadfish, gut absorption kinetics, 399
  liver, elongation factor 1 in, 422
  liver, leucine transport and temperature in, 424
  liver protein synthetic parameters, effect of leucine on, 412
  otic labyrinth, afferent activity in, 414
  plasma protein synthesis in, 414
  sonic muscle, calcium binding sites in synaptic vesicles of, 412
  swimbladder, secretion of inert gas into, 434
Transglutaminase activity, in human erythrocytes, 419
Transmitter uptake, by squid brain synaptosomes, 400
TREMBLAY, G. C. See J. F. McCarthy, 538
Tridacna, function of gills in, 331
TROLL, W. See J. G. Csernansky, 406; E. S. Harris, 412
Trout erythrocytes, permeability to nonelectrolytes, 322
TROXLER, R. F. See J. C. Cosner, 405
Tryptophan photoproducts, in dogfish, 436
Tubipora musica, circadian rhythms in, 236
Tubulin, dogfish brain, helical ribbons in, 417
Tunicate, oxygen uptake of, 297
TURGEON, K. W. Osmotic adjustment in an estuarine population of *Urosalpinx cinerea* (Say, 1822) (Muricidae, Gastropoda), 601
TWEEDELL, K. S. Utilization of ^{3}H-thymidine triphosphate by developing stages of *Pectinaria gouldii*, 260

U

Ultrastructure, of *Astropecten scoparius*, 560
  of *Daphnia*, 370
  of foraminifera, 437
  of histone genes, in sea urchin, 435
  of *Histriobdella homari*, 489
  of hydrozoans, 214
  of mouse eggs, 435
  of muscles, in *Aedes aegypti*, 283
  of *Nitella* endoplasmic filaments, 398
  of postsynaptic membrane of frog muscle after collagenase digestion, 430
  of Schwann cell sheath, in giant axon, 402
Ulva, diurnal variation of photosynthesis in, 420
Uptake, of oxygen, in *Styela plicata*, 297
Urea, permeability of trout erythrocytes to, 322
Urease, from lugworm, 96
Urosalpinx, osmotic adjustment in, 601

V

Veliger larvae, ciliary activity in, 182
Venom, brown widow spider, in synaptic vesicles of frog muscle, 406
Vesicles, adrenergic, 428
Voltage clamp currents, from squid giant axons, 430
VRANCKX, R. See M. Durliat, 467

W

WAGGONER, A. S. See A. Grinvald, 411
WALTON, A. J. See E. G. Ruby, 428
WANG, C. H. See A. Grinvald, 411
Water currents, and suspension feeding, 344
Water transport, in bivalve gills, 331
WEIR, G. C. See B. D. Noe, 422
WEISSMANN, G. See P. Dunham, 407
WEISSMANN, L. B. See L. Lorand, 419
WESTHEAD, E. W. See D. B. Sattelle, 428
WHITLATCH, R. B. Dispersion patterns of spionid polychaetes at Barnstable Harbor, Massachusetts, 433
WHITTAKER, J. R. Melanogenesis in melanocytes of ascidian embryos: quantity is predetermined independently of cell size and number of nuclei, 434
WILNER, A. N. See A. B. DuBois, 407
WILSON, D. R. See J. K. Haynes, 413

WITTENBERG, D. See J. Wittenberg, 434
WITTENBERG, J., W. WITTENBERG AND D. WITTENBERG. Roles of blood plasma and erythrocytes in secretion of inert gas into the teleost swimbladder, 434
WITTENBERG, W. See J. Wittenberg, 434
WOLF, D. P., S. V. NICOSIA AND L. MASTROIANNI, JR. Surface characteristics of zona-free mouse eggs before and after insemination, 435
WOOD, T. See B. J. PETERSON, 424

X

Xenopus laevis, localization of translatable RNA in, 424

Y

YULO, T. See S. Zigman, 436

Z

ZDUNSKI, D., B. HAMKALO AND L. KEDES. Actively transcribing chromatin of sea urchin (*A. punctulata*) visualized by electron microscopy, 435
ZERIAL, A. See P. Dunham, 407
ZIGMAN, S. AND T. YULO. Aspects of biological effects of tryptophan photoproducts, 436

Continued from Cover Two

4. ***Literature Cited.*** The list of references should be headed LITERATURE CITED, should conform in punctuation and arrangement to the style of recent issues of THE BIOLOGICAL BULLETIN, and must be typed *double-spaced* on separate pages. Note that citations should include complete titles and inclusive pagination. Journal abbreviations should normally follow those of the U. S. A. Standards Institute (USASI), as adopted by BIOLOGICAL ABSTRACTS and CHEMICAL ABSTRACTS, with the minor differences set out below. The most generally useful list of biological journal titles is that published each year by BIOLOGICAL ABSTRACTS of those abstracted (most recent issue: November, 1972). Foreign authors, and others who are accustomed to use THE WORLD LIST OF SCIENTIFIC PERIODICALS, may find a booklet published by the Biological Council of the U.K. (obtainable from the Institute of Biology, 41 Queen's Gate, London, S.W.7, England, U.K. at £0.65 or $1.75) useful, since it sets out the WORLD LIST abbreviations for most biological journals with notes of the USASI abbreviations where these differ. CHEMICAL ABSTRACTS publishes quarterly supplements of additional abbreviations. The following points of reference style for THE BIOLOGICAL BULLETIN differ from USASI (or modified WORLD LIST) usage:

A. Journal abbreviations, and book titles, all underlined (for *italics*)

B. All components of abbreviations with initial capitals (not as European usage in WORLD LIST *e.g. J. Cell. Comp. Physiol.* NOT *J. cell. comp. Physiol.*)

C. *All* abbreviated components must be followed by a period, whole word components *must not* (not strictly as USASI usage, *i.e. J. Cancer Res.*)

D. Space between all components (*e.g. J. Cell. Comp. Physiol.* not *J.Cell.Comp.Physiol.*)

E. We strongly recommend that more unusual words in journal titles be spelled out in full, rather than employing lengthy, peculiar "abbreviations" or new abbreviations invented by the author. For example, use *Rit Visindafjélags Islendinga* without abbreviation. Even in more familiar languages, *Z. Vererbungslehre* is preferred to *Z. VererbLehre* (WORLD LIST) or *Z. Vererbungsl.* (USASI). *Accurate and complete communication of the reference is more important than minor savings in printing costs.*

F. All single word journal titles in full (*e.g. Veliger, Ecology, Brain*).

G. The order of abbreviated components should be the same as the word order of the complete title (*i.e. Proc.* and *Trans.* placed where they appear, not transposed as in some BIOLOGICAL ABSTRACTS listings).

H. Spell out *London, Tokyo, Paris, Edinburgh, Lisbon, etc.* where part of journal title.

I. Series letters *etc.* immediately before volume number.

J. A few well-known international journals in their preferred forms rather than WORLD LIST or USASI usage (*e.g. Nature, Science, Evolution* NOT *Nature, Lond.; Science, N.Y.; Evolution, Lancaster, Pa.*)

K. The correct abbreviation for THE BIOLOGICAL BULLETIN is *Biol. Bull.*

5. ***Figures.*** The dimensions of the printed page, 5 by 7⅜ inches, should be kept in mind in preparing figures for publication. Illustrations should be large enough so that all details will be clear after appropriate reduction. Explanatory matter should be included separately in legends as far as possible, although the axes should always be numbered and identified on the illustration itself. Figures should be prepared for reproduction as either line-cuts or halftones; no other methods will be used. Figures to be reproduced as line-cuts should be drawn in black ink on white paper, good quality tracing cloth or plastic, or blue-lined coordinate paper; those to be reproduced as halftones should be mounted on board, and both designating numbers or letters and scale-bars should be affixed directly on the figures. We recommend that halftones submitted to us be mounted prints made at about 1½ times the linear dimensions of the final printing desired (the actual best reductions are achieved from copy in the range from 1¼ to 2 times the linear dimensions). As regards line-blocks, originals can be designed for even greater reductions but are best in the range 1½ to 3 times. *All* figures should be numbered in consecutive order, with no distinction between text and plate-figures. The author's name should appear on the reverse side of all figures, and the inked originals for line-blocks must be submitted for block-making.

6. ***Mailing.*** Manuscripts should be packed flat. All illustrations larger than 8½ by 11 inches must be accompanied by photographic reproductions or tracings that may be folded to page size.

Reprints. Reprints may be obtained at cost; approximate prices will be furnished by the Managing Editor upon request.

CONTENTS

ANDERSON, O. ROGER AND ALLAN W. H. BÉ
A cytochemical fine structure study of phagotrophy in a planktonic foraminifer, *Hastigerina pelagica* (d'Orbigny) 437

DURAND, JACQUES P.
Ocular development and involution in the European cave salamander, *Proteus anguinus* Laurenti 450

DURLIAT, MICHELE AND ROGER VRANCKX
Coagulation in the crayfish, *Astacus leptodactylus:* attempts to identify a fibrinogen-like factor in the hemolymph 467

HOFFMANN, RICHARD J.
Genetics and asexual reproduction of the sea anemone *Metridium senile* 478

JENNINGS, J. B. AND S. R. GELDER
Observations on the feeding mechanism, diet and digestive physiology of *Histriobdella homari* van Beneden 1858: an aberrant polychaete symbiotic with North American and European lobsters 489

KASTENDIEK, JON
Behavior of the sea pansy *Renilla kollikeri* Pfeffer (Coelenterata: Pennatulacea) and its influence on the distribution and biological interactions of the species 518

MCCARTHY, J. F., A. N. SASTRY AND G. C. TREMBLAY
Thermal compensation in protein and RNA synthesis during the intermolt cycle of the American lobster, *Homarus americanus* 538

MITTON, JEFFRY B. AND RICHARD K. KOEHN
Morphological adaptation to thermal stress in a marine fish, *Fundulus heteroclitus* 548

OGURO, CHITARU, MIÉKO KOMATSU AND YASUO T. KANO
Development and metamorphosis of the sea star, *Astropecten scoparius* Valenciennes 560

RUBY, E. G. AND K. H. NEALSON
Symbiotic association of *Photobacterium fischeri* with the marine luminous fish *Monocentris japonica:* a model of symbiosis based on bacterial studies 574

SMITH, RALPH I.
Exchanges of sodium and chloride at low salinities by *Nereis diversicolor* (Annelida, Polychaeta) 587

TURGEON, KENNETH W.
Osmotic adjustment in an estuarine population of *Urosalpinx cinerea* (Say, 1822) (Muricidae, Gastropoda) 601

INDEX TO VOLUME 151 615

MBL/WHOI LIBRARY
WH 1B1N %